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(54) Title: COMBINATORIAL LIBRARIES OF PROTEINS HAVING THE SCAFFOLD STRUCTURE OF C-TYPE LECTIN-LIKE DOMAINS

(57) Abstract: A novel family of protein libraries comprising CTLDS (C-type Lectin-Like Domains) in which internal polypeptide loop-regions lining the ligand binding sites in CTLDs have been replaced with ensembles of completely or partially randomised polypeptide segments. Tetranectin CTLDs were chosen as framework for the preferred embodiment of the invention; and versatile phagemid vectors useful in the generation and manipulation of human and murine tetranectin CTLD libraries are disclosed as part of this invention. Tetranectin CTLDs in monomeric as well as in trimeric form are efficiently displayed as gene III fusions in fully functional form by the recombinant fd phage display vector. CTLD derivatives with affinity for new ligands may readily be isolated from libraries of vectors displaying CTLDs, in which loop-regions have been randomised, using one or more rounds of enrichment by screening or selection followed by amplification of the enriched subpopulation in each round. The efficiency with which protein products containing CTLDs with new binding properties can be produced, e.g. by bacterial expression in *in vitro* refolding, in mono-, tri-, or multimeric formats provides important advantages in terms of simplicity, cost and efficiency of generation, production and diagnostic or therapeutic applications in comparison to recombinant antibody derivatives.

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Combinatorial libraries of proteins having the scaffold structure of C-type lectin-like domains

FIELD OF THE INVENTION

This invention describes a system which relates to the generation of randomised libraries of ligand-binding protein units derived from proteins containing the so-called C-type lectin like domain (CTLD) of which the carbohydrate recognition domain (CRD) of C-type lectins represents one example of a family of this protein domain.

BACKGROUND OF THE INVENTION

The C-type lectin-like domain (CTLD) is a protein domain family which has been identified in a number of proteins isolated from many animal species (reviewed in Drickamer and Taylor (1993) and Drickamer (1999)). Initially, the CTLD domain was identified as a domain common to the so-called C-type lectins (calcium-dependent carbohydrate binding proteins) and named "Carbohydrate Recognition Domain" ("CRD"). More recently, it has become evident that this domain is shared among many eukaryotic proteins, of which several do not bind sugar moieties, and hence, the canonical domain has been named as CTLD.

CTLDs have been reported to bind a wide diversity of compounds, including carbohydrates, lipids, proteins, and even ice [Aspberg et al. (1997), Bettler et al. (1992), Ewart et al. (1998), Graversen et al. (1998), Mizumo et al. (1997), Sano et al. (1998), and Tormo et al. (1999)]. Only one copy of the CTLD is present in some proteins, whereas other proteins contain from two to multiple copies of the domain. In the physiologically functional unit multiplicity in the number of CTLDs is often achieved by assembling single copy protein protomers into larger structures.

The CTLD consists of approximately 120 amino acid residues and, characteristically, contains two or three intra-chain disulfide bridges. Although the similarity at the amino acid sequence level between CTLDs from different proteins is relatively low, the 3D-structures of a number of CTLDs have been found to be highly conserved, with the structural variability essentially confined to a so-called loop-region, often defined by up to five loops. Several CTLDs contain either one or two binding sites for calcium and most of the side chains which interact with calcium are located in the loop-region.

On the basis of CTLDs for which 3D structural information is available, it has been inferred that the canonical CTLD is structurally characterised by seven main secondary-structure elements (i.e. five β-strands and two α-helices) sequentially appearing in the order β1; α1; α2; β2; β3; β4; and β5 (Fig. 1, and references given therein). In all CTLDs, for which 3D structures have been determined, the β-strands are arranged in two anti-parallel β-sheets, one composed of β1 and β5, the other composed of β2, β3 and β4. An additional β-strand, β0, often precedes β1 in the sequence and, where present, forms an additional strand integrating with the β1, β5-sheet. Further, two disulfide bridges, one connecting α1 and β5 (C_I-C_{IV}, Fig. 1) and one connecting β3 and the polypeptide segment connecting β4 and β5 (C_{II}-C_{III}, Fig. 1) are invariantly found in all CTLDs characterised so far. In the CTLD 3D-structure, these conserved secondary structure elements form a compact scaffold for a number of loops, which in the present context collectively are referred to as the "loop-region", protruding out from the core. These loops are in the primary structure of the CTLDs organised in two segments, loop segment A, LSA, and loop segment B, LSB. LSA represents the long polypeptide segment connecting β2 and β3 which often lacks regular secondary structure and contains up to four loops. LSB

represents the polypeptide segment connecting the β-strands β3 and β4. Residues in LSA, together with single residues in β4, have been shown to specify the Ca^{2+}- and ligand-binding sites of several CTLDs, including that of tetranectin. E.g. mutagenesis studies, involving substitution of single or a few residues, have shown, that changes in binding specificity, Ca^{2+}-sensitivity and/or affinity can be accommodated by CTLD domains [Weis and Drickamer (1996), Chiba et al. (1999), Graversen et al. (2000)].

As noted above, overall sequence similarities between CTLDs are often limited, as assessed e.g. by aligning a prospective CTLD sequence with the group of structure-characterized CTLDs presented in Fig. 1, using sequence alignment procedures and analysis tools in common use in the field of protein science. In such an alignment, typically 22-30% of the residues of the prospective CTLD will be identical with the corresponding residue in at least one of the structure-characterized CTLDs. The sequence alignment shown in Fig. 1 was strictly elucidated from actual 3D structure data, so the fact that the polypeptide segments of corresponding structural elements of the framework also exhibit strong sequence similarities provide a set of direct sequence-structure signatures, which can readily be inferred from the sequence alignment.

The implication is that also CTLDs, for which precise 3D structural information is not yet available, can nonetheless be used as frameworks in the construction of new classes of CTLD libraries. The specific additional steps involved in preparing starting materials for the construction of such a new class of CTLD library on the basis of a CTLD, for which no precise 3D structure is available, would be the following: (1) Alignment of the sequence of the new CTLD with the sequence shown in Fig. 1; and (2) Assignment of approximate locations of framework structural elements

as guided by the sequence alignment, observing any requirement for minor adjustment of the alignment to ensure precise alignment of the four canonical cysteine residues involved in the formation of the two conserved disulfide bridges (C_I-C_{IV} and C_{II}-C_{III}, in Fig. 1). The main objective of these steps would be to identify the sequence location of the loop-region of the new CTLD, as flanked in the sequence by segments corresponding to the β2-, β3- and β4-strands. To provide further guidance in this the results of an analysis of the sequences of 29 bona fide CTLDs are given in Table 1 below in the form of typical tetrapeptide sequences, and their consensus sequences, found as parts of CTLD β2- and β3-strands, and the precise location of the β4-strand by position and sequence characteristics as elucidated.

Table 1: β2, β3 and β4 consensus elements analysis

CTLD	β2	---	LSA	---	β3	LSB	β4
IX-A	WIGLRW---QGKVKQCNSEWSDGSSVS--YENWIE---------AESKT----------CLGLEKETDFRKWVNIYC
MGL	WIGLTDQ--NGP--WRWVDGTDFEKGFKNWAP---------LQPDNWFGHGLGGGEDCAHITTG--GFWNDDVC
LIT	WIGLHDPKKNRR--WHWSSGSLVS--YKSWGI---------GAPSSVNP-----GY-CVSLTSSTGFQKWKDVPC
CHL	WIGLTDENQEGE--WQWVDGTDTRSSFTFWKE---------GEPNNRGF-----NEDCAHVWTS--GQWNDVYC
IGE-FCR	WIGLRNLDLKGEFIWV--DGSHVD--YSNWAP---------GEPTSRSQ-----GEDCVMMRGS--GRWNDAFC
TCL-1	WIGLTDKDSEGT--WKWVDGTPLT--TAFWST---------DEPNDGAVN----GEDCVSLYYHTQPEFKNWNDLAC
KUCR	WIGLTDQGTEGN--WRWVDGTPFDYVQSRRFWRK---------GQPDWRHGNGE--REDCVHLQ-----RMWNDMAC
CD94	WIGLSYSEEHTA--WLWENGSALSQ-YLSFET---------FNTKN---------CIAYNPN--GNALDESC
CPCP	WIGLNDRTIEGDFRWS--DGHPMQ--FENWRP---------NQPDNFFAA----GEDCVVMIWHEKGEWNDVPC
PAP	WIGLHDPTQGTEPNGEG-WEWSSSDVMN--YFAWER---------N-PSTISSPGH-----CASLSRSTAFLRWKDYNC
NEU	WIGLNDRIVEQD--FQWTDNTGLQ--YENWRE---------NQPDNFFAG----GEDCVVLVSHEIGKWNDVPC
ESL	WIGIRKVNNV----WVW-VGTQKPLTEEAKNWAP---------GEPNNRQK-----DEDCVEIYIKREKDVGMWNDERC
NKg2A	WIGVFRNSSHHP--WVTMNGLAFKHEIKDSDNA---------ELNCAVLQV----NRLKSAQC
GP120	WMGLSDLNQEGT--WQWVDGSPLLPS-FKQYWNR---------GEPNNVG-----EEDCAEFSGN--G-WNDDKC
MMR	WIGLFRNV-EGT--WLWINNSPVS--FVNWNT---------GDPSGE---------RNDCVALHASS-GFWSNIHC
TN	WLGLNDMAAEGT----WVDMTGARIAYKNWETEIT------AQPDGGK-------TENCAVLSGAANGKWFDKRC
SCGF	WLGVHDRRAEGL--YLFENGQRVS--FFAWHRSPRPELGAQPSASPHPLSPDQPNGGGT-------LENCVAQASDD-GSWWDHDC
PLC	WLGASDLNIEGR--WLW-EGQRRMN-YTNWSP---------GQPDNAGG-----IEHCLELRRDLGNYLWNDYQC
H1-ASR	WMGLHD--QNGP--WKWVDGTDYETGFKNWRP---------EQPDDWYGHGLGGGEDCAHFTDD--GRWNDDVC
IX-B	WMGLSNVWNQCN--WQWSNAAMLR--YKAWAE---------ESY----------CVYFKSTN-NKWRSRAC
LY49A	WVGLSYDNKKKD--WAWIDNRPSKLALNTRKY---------NIRDGG----------CMLLSKT---RLDNGNC
TU14	WVGADN-LQDGAYNFNWNDGVSLPTDSDLWSP---------NEPSNPQSWQL----CVQIWSKY-NLLDDVGC
rSP-A	YLGMIEDQTPGD--FHYLDGASVH--YTNWYP---------GEPIGQG-------KEKCVEMYTD--GTWNDRGC
BCON	YLSMNDISTEGR--FTYPTGEILV--YSNWAD---------GEPNNSDEGQ---PENCVEIFPD--GKWNDVPC
BCL43	YLSMNDISKEGK--FTYPTGGSLD--YSNWAP---------GEPNNRAKDEG--PENCLEIYSD--GNWNDIEC
MBP-A	FLGITDEVTEGQ--FMYVTGGRLT--YSNWKK---------DEPNDHGS-----GEDCVTIVDN--GLWNDISC
SP-D	FLSMTDSKTEGK--FTYPTGESLV--YSNWAP---------GEPNDDGG-----SEDCVEIFTN--GKWNDRAC
CL-L1	FIGVNDLERGQ--YMFTDNTPLQN-YSNWNE---------GEPSDPYG-----HEDCVEMLSS--GRWNDTEC
DCIR	FVGLSDP--EGQRHWQWVDQTP-----YNESSTFWHP---------REPSDPN---------ERCVVLNFRKSPKRWG-WNDVNC

Notes: LSA, Loop Segment A; LSB, Loop Segemnt B.

Sequences taken from: Berglund and Petersen (1992) [TN, tetranectin]; Bertrand et al. (1996) [LIT, lithostatin]; Mann et al. (2000) [MGL, mouse macrophage galactose lectin, KUCR, Kupffer cell receptor, NEU, chicken neurocan, PLC, perlucin, H1-ASR, asialoglycoprotein receptor]; Mio et al. (1998) [CPCP, cartilage proteoglycan core protein, IGE-FCR, IgE Fc receptor, PAP, pancreatitis-associated protein, MMR, mouse macrophage receptor, NKG2, Natural Killer group, SCGF, stem cell growth factor]; Mizuno et al. (1997) [IX-A and B, factor IX/X binding protein, MBP, mannose binding protein]; Ohtani et al. (1999) [BCON, bovine conglutinin, BCL43, bovine CL43, CL-L1, collectin liver 1, SP-A, surfactant protein A, SP-D, surfactant protein D]; Poget et al. (1999) [ESL, e-selectin, TU14, tunicate c-type lectin]; Tormo et al. (1999) [CD94,CD94 NK receptor domain, LY49A, LY49A NK receptor domain]; Zhang et al. (2000) [CHL, chicken hepatic lectin, TCL-1, trout c-type lectin, GP120, HIV gp 120-binding c-type lectin, DCIR, dendritic cell immuno receptor]

Of the 29 β2-strands,

14 were found to conform to the consensus sequence WIGX (of which 12 were WIGL sequences, 1 was a WIGI sequence and 1 was a WIGV sequence);

3 were found to conform to the consensus sequence WLGX (of which 1 was a WLGL sequence, 1 was a WLGV sequence and 1 was a WLGA sequence);

3 were found to be WMGL sequences;

3 were found to conform to the consensus sequence YLXM (of which 2 were YLSM sequences and 1 was an YLGM sequence);

2 were found to conform to the consensus sequence WVGX (of which 1 was a WVGL sequence and 1 was a WVGA sequence); and

the sequences of the remaining 4 β2-strands in the collection were FLGI, FVGL, FIGV and FLSM sequences, respectively.

Therefore, it is concluded that the four-residue β2 consensus sequence ("β2cseq") may be specified as follows:

Residue 1: An aromatic residue, most preferably Trp, less preferably Phe and least preferably Tyr.

Residue 2: An aliphatic or non-polar residue, most preferably Ile, less preferably Leu or Met and least preferably Val.

Residue 3: An aliphatic or hydrophilic residue, most preferably Gly and least preferably Ser.

Residue 4: An aliphatic or non-polar residue, most preferably Leu and less preferably Met, Val or Ile.

Accordingly the β2 consensus sequence may be summarized as follows:

β2cseq: (W,Y,F)-(I,L,V,M)-(G,S)-(L,M,V,I),

where the underlined residue denotes the most commonly found residue at that sequence position.

All 29 β3-strands analysed are initiated with the Cys_{II} residue canonical for all known CTLD sequences, and of the 29 β3-strands,

5 were found to conform to the consensus sequence CVXI (of which 3 were CVEI sequences, 1 was a CVTI sequence and 1 was a CVQI sequence);

4 were found to conform to the consensus sequence CVXM (of which 2 were CVEM sequences, 1 was a CVVM sequence and 1 was a CVMM sequence);

6 were found to conform to the consensus sequence CVXL (of which 2 were CVVL sequences, 2 were a CVSL sequence, 1 was a CVHL sequence and 1 was CVAL sequence);

3 were found to conform to the consensus sequence CAXL (of which 2 were CAVL sequences and 1 was a CASL sequence);

2 were found to conform to the consensus sequence CAXF (of which 1 was 1 CAHF sequence and 1 was a CAEF sequence);

2 were found to conform to the consensus sequence CLXL (of which 1 was a CLEL sequence and 1 was a CLGL sequence); and

the sequences of the remaining 7 β3-strands in the collection were CVYF, CVAQ, CAHV, CAHI, CLEI, CIAY, and CMLL sequences, respectively.

Therefore, it is concluded that the four-residue β3 consensus sequence ("β3cseq") may be specified as follows:

Residue 1: Cys, being the canonical Cys_{II} residue of CTLDs

Residue 2: An aliphatic or non-polar residue, most preferably Val, less preferably Ala or Leu and least preferably Ile or Met

Residue 3: Most commonly an aliphatic or charged residue, which most preferably is Glu

Residue 4: Most commonly an aliphatic, non-polar, or aromatic residue, most preferably Leu or Ile, less preferably Met or Phe and least preferably Tyr or Val.

Accordingly the β3 consensus sequence may be summarized as follows:

β3cseq: (C)-(V,A,L,I,M)-(E,X)-(L,I,M,F,Y,V),

where the underlined residue denotes the most commonly found residue at that sequence position.

It is observed from the known 3D-structures of CTLDs (Fig. 1), that the β4-strands most often are comprised by five residues located in the primary structure at positions -6 to -2 relative to the canonical Cys_{III} residue of all known

CTLDs, and less often are comprised by four residues located at positions -5 to -2 relative to the canonical Cys_{III} residue of all known CTLDs. The residue located at position -3, relative to Cys_{III}, is involved in co-ordination of the site 2 calcium ion in CTLDs housing this site, and this notion is reflected in the observation, that of the 29 CTLD sequences analysed in Table 1, 27 have an Asp-residue or an Asn-residue at this position, whereas 2 CTLDs have a Ser at this position. From the known CTLD 3D-structures it is also noted, that the residue located at position -5, relative to the Cys_{III} residue, is involved in the formation of the hydrophobic core of the CTLD scaffold. This notion is reflected in the observation, that of the 29 CTLD sequences analysed 25 have a Trp-residue, 3 have a Leu-residue, and 1 an Ala-residue at this position. 18 of the 29 CTLD sequences analysed have an Asn-residue at position -4. Further, 19 of the 29 β4-strand segments are preceded by a Gly residue.

Of the 29 central three residue motifs located at positions -5, -4 and -3 relative to the canonical Cys_{III} residue in the β4-strand:

22 were of the sequence WXD (18 were WND, 2 were WKD, 1 was WFD and 1 was WWD),

2 were of the sequence WXN (1 was WVN and 1 was WSN),

and the remaining 5 motifs (WRS, LDD, LDN, LKS and ALD) were each represented once in the analysis.

It has now been found that each member of the family of CTLD domains represents an attractive opportunity for the construction of new protein libraries from which members with affinity for new ligand targets can be identified and isolated using screening or selection methods. Such libraries may be constructed by combining a CTLD framework struc-

ture in which the CTLD's loop-region is partially or completely replaced with one or more randomised polypeptide segments.

One such system, where the protein used as scaffold is tetranectin or the CTLD domain of tetranectin, is envisaged as a system of particular interest, not least because the stability of the trimeric complex of tetranectin protomers is very high (International Patent Application Publication No. WO 98/56906 A2).

Tetranectin is a trimeric glycoprotein [Holtet et al. (1997), Nielsen et al. (1997)], which has been isolated from human plasma and found to be present in the extracellular matrix in certain tissues. Tetranectin is known to bind calcium, complex polysaccharides, plasminogen, fibrinogen/fibrin, and apolipoprotein (a). The interaction with plasminogen and apolipoprotein (a) is mediated by the so-called kringle 4 protein domain therein. This interaction is known to be sensitive to calcium and to derivatives of the amino acid lysine [Graversen et al. (1998)].

A human tetranectin gene has been characterised, and both human and murine tetranectin cDNA clones have been isolated. Both the human and the murine mature protein comprise 181 amino acid residues (Fig. 2). The 3D-structures of full length recombinant human tetranectin and of the isolated tetranectin CTLD have been determined independently in two separate studies [Nielsen et al. (1997) and Kastrup et al. (1998)]. Tetranectin is a two- or possibly three-domain protein, i.e. the main part of the polypeptide chain comprises the CTLD (amino acid residues Gly53 to Val181), whereas the region Leu26 to Lys52 encodes an alpha-helix governing trimerisation of the protein via the formation of a homotrimeric parallel coiled coil. The polypeptide segment Glu1 to Glu25 contains the binding site for

complex polysaccharides (Lys6 to Lys15) [Lorentsen et al. (2000)] and appears to contribute to stabilisation of the trimeric structure [Holtet et al. (1997)]. The two amino acid residues Lys148 and Glu150, localised in loop 4, and Asp165 (localised in β4) have been shown to be of critical importance for plasminogen kringle 4 binding, whereas the residues Ile140 (in loop 3) and Lys166 and Arg167 (in β4) have been shown to be of some importance [Graversen et al. (1998)]. Substitution of Thr149 (in loop 4) with an aromatic residue has been shown to significantly increase affinity of tetranectin to kringle 4 and to increase affinity for plasminogen kringle 2 to a level comparable to the affinity of wild type tetranectin for kringle 4 [Graversen et al. (2000)].

OBJECT OF THE INVENTION

The object of the invention is to provide a new practicable method for the generation of useful protein products endowed with binding sites able to bind substance of interest with high affinity and specificity.

The invention describes one way in which such new and useful protein products may advantageously be obtained by applying standard combinatorial protein chemistry methods, commonly used in the recombinant antibody field, to generate randomised combinatorial libraries of protein modules, in which each member contains an essentially common core structure similar to that of a CTLD.

The variation of binding site configuration among naturally occurring CTLDs shows that their common core structure can accommodate many essentially different configurations of the ligand binding site. CTLDs are therefore particularly well suited to serve as a basis for constructing such new and useful protein products with desired binding properties.

In terms of practical application, the new artificial CTLD protein products can be employed in applications in which antibody products are presently used as key reagents in technical biochemical assay systems or medical *in vitro* or *in vivo* diagnostic assay systems or as active components in therapeutic compositions.

In terms of use as components of *in vitro* assay systems, the artificial CTLD protein products are preferable to antibody derivatives as each binding site in the new protein product is harboured in a single structurally autonomous protein domain. CTLD domains are resistant to proteolysis, and neither stability nor access to the ligand-binding site is compromised by the attachment of other protein domains to the N- or C-terminus of the CTLD. Accordingly, the CTLD binding module may readily be utilized as a building block for the construction of modular molecular assemblies, e.g. harbouring multiple CLTDs of identical or nonidentical specificity in addition to appropriate reporter modules like peroxidases, phosphatases or any other signal-mediating moiety.

In terms of *in vivo* use as essential component of compositions to be used for *in vivo* diagnostic or therapeutic purposes, artificial CTLD protein products constructed on the basis of human CTLDs are virtually identical to the corresponding natural CTLD protein already present in the body, and are therefore expected to elicit minimal immunological response in the patient. Single CTLDs are about half the mass of the smallest functional antibody derivative, the single-chain Fv derivative, and this small size may in some applications be advantageous as it may provide better tissue penetration and distribution, as well as a shorter half-life in circulation. Multivalent formats of CTLD proteins, e.g. corresponding to the complete tetranectin trimer or the further multimerized collectins, like e.g.

mannose binding protein, provide increased binding capacity and avidity and longer circulation half-life.

One particular advantage of the preferred embodiment of the invention, arises from the fact that mammalian tetranectins, as exemplified by murine and human tetranectin, are of essentially identical structure. This conservation among species is of great practical importance as it allows straightforward swapping of polypeptide segments defining ligand-binding specificity between e.g. murine and human tetranectin derivatives. The option of facile swapping of species genetic background between tetranectin derivatives is in marked contrast to the well-known complications of effecting the "humanisation" of murine antibody derivatives.

Further advantages of the invention are:

The availability of a general and simple procedure for reliable conversion of an initially selected protein derivative into a final protein product, which without further reformatting may be produced in bacteria (e.g. *Escherichia coli*) both in small and in large scale (International Patent Application Publication No. WO 94/18227 A2).

The option of including several identical or non-identical binding sites in the same functional protein unit by simple and general means, thereby enabling the exploitation even of weak affinities by means of avidity in the interaction, or the construction of bi- or heterofunctional molecular assemblies (International Patent Application Publication No. WO 98/56906 A2).

The possibility of modulating binding by addition or removal of divalent metal ions (e.g. calcium ions) in combinational libraries with one or more preserved metal binding site(s) in the CTLDs.

SUMMARY OF THE INVENTION

The present invention provides a great number of novel and useful proteins each being a protein having the scaffold structure of C-type lectin-like domains (CTLD), said protein comprising a variant of a model CTLD wherein the α-helices and β-strands and connecting segments are conserved to such a degree that the scaffold structure of the CTLD is substantially maintained, while the loop region is altered by amino acid substitution, deletion, insertion or any combination thereof, with the proviso that said protein is not any of the known CTLD loop derivatives of C-type lectin-like proteins or C-type lectins listed in the following Table 2.

TABLE 2: Known β2, β3, β4, LSA and LSB CTLD derivatives

Table 2A: LSA derivatives (β2 and β3 consensus elements are underlined)

CTLD	Mut.	LSA sequence (one letter code)	Reference
hTN	TND116A	WLGLNAMAAEGTWVDMTGARIAYKNWETEITAQPDGGKTENCAVL	Graversen et al. (1998)
	TNE120A	WLGLNDMAAAGTWVDMTGARIAYKNWETEITAQPDGGKTENCAVL	Graversen et al. (1998)
	TNK134A	WLGLNDMAAEGTWVDMTGARIAYANWETEITAQPDGGKTENCAVL	Graversen et al. (1998)
	TNI140A	WLGLNDMAAEGTWVDMTGARIAYKNWETEATAQPDGGKTENCAVL	Graversen et al. (1998)
	TNQ143A	WLGLNDMAAEGTWVDMTGARIAYKNWETEITAAPDGGKTENCAVL	Graversen et al. (1998)
	TND145A	WLGLNDMAAEGTWVDMTGARIAYKNWETEITAQPAGGKTENCAVL	Graversen et al. (1998)
	TNK148A	WLGLNDMAAEGTWVDMTGARIAYKNWETEITAQPDGGATENCAVL	Graversen et al. (1998)
	TNK148M	WLGLNDMAAEGTWVDMTGARIAYKNWETEITAQPDGGMTENCAVL	Graversen et al. (2000)
	TNK148R	WLGLNDMAAEGTWVDMTGARIAYKNWETEITAQPDGGRTENCAVL	Graversen et al. (2000)
	TNT149F	WLGLNDMAAEGTWVDMTGARIAYKNWETEITAQPDGGKFENCAVL	Graversen et al. (2000)
	TNT149M	WLGLNDMAAEGTWVDMTGARIAYKNWETEITAQPDGGKMENCAVL	Graversen et al. (2000)
	TNT149R	WLGLNDMAAEGTWVDMTGARIAYKNWETEITAQPDGGKRENCAVL	Graversen et al. (2000)
	TNT149Y	WLGLNDMAAEGTWVDMTGARIAYKNWETEITAQPDGGKYENCAVL	Graversen et al. (2000)
	TNE150A	WLGLNDMAAEGTWVDMTGARIAYKNWETEITAQPDGGKTANCAVL	Graversen et al. (1998)
	TNE150D	WLGLNDMAAEGTWVDMTGARIAYKNWETEITAQPDGGKTDNCAVL	Graversen et al. (2000)
	TNE150Q	WLGLNDMAAEGTWVDMTGARIAYKNWETEITAQPDGGKTQNCAVL	Graversen et al. (2000)
	TNN151A	WLGLNDMAAEGTWVDMTGARIAYKNWETEITAQPDGGKTEACAVL	Graversen et al. (1998)
	TNK148R,T149Y	WLGLNDMAAEGTWVDMTGARIAYKNWETEITAQPDGGRYENCAVL	Graversen et al. (2000)
	TNT149Y,E150Q	WLGLNDMAAEGTWVDMTGARIAYKNWETEITAQPDGGKYQNCAVL	Graversen et al. (2000)
	TNT149Y,D165N	WLGLNDMAAEGTWVDMTGARIAYKNWETEITAQPDGGKYENCAVL	Graversen et al. (2000)
rMBP	QPD	FLGITDEVTEGQFMYVTGGRLTYSNWKKDQPDDHGSGEDCVTI	Drickamer (1992)
	N187D	FLGITDEVTEGQFMYVTGGRLTYSNWKKDEPDDHGSGEDCVTI	Iobst et al. (1994)
	H189A	FLGITDEVTEGQFMYVTGGRLTYSNWKKDEPNDAGSGEDCVTI	Iobst et al. (1994)
	H189G	FLGITDEVTEGQFMYVTGGRLTYSNWKKDEPNDGGSGEDCVTI	Iobst et al. (1994)
	QPDW	FLGITDEVTEGQFMYVTGGRLTYSNWKKDQPDDWGSGEDCVTI	Iobst & Drickamer (1994)
	QPDWG	FLGITDEVTEGQFMYVTGGRLTYSNWKKDQPDDWYGHGLGGGEDCVTI	Iobst & Drickamer (1994)
	QPDWG/Y/A	FLGITDEVTEGQFMYVTGGRLTYSNWKKDQPDDWAGHGLGGGEDCVTI	Iobst & Drickamer (1994)

QPDWG/Y/Q	FLGITDEVTEGQFMYVTGGRLTYSNWKKDQPDDWQGHGLGGGEDCVTI	Iobst & Drickamer (1994)
QPDWG/G/A	FLGITDEVTEGQFMYVTGGRLTYSNWKKDQPDDWYAHGLGGGEDCVTI	Iobst & Drickamer (1994)
QPDWG/H/A	FLGITDEVTEGQFMYVTGGRLTYSNWKKDQPDDWYGAGLGGGEDCVTI	Iobst & Drickamer (1994)
QPDWG/H/Q	FLGITDEVTEGQFMYVTGGRLTYSNWKKDQPDDWYGQGLGGGEDCVTI	Iobst & Drickamer (1994)
QPDWG/H/E	FLGITDEVTEGQFMYVTGGRLTYSNWKKDQPDDWYGEGLGGGEDCVTI	Iobst & Drickamer (1994)
QPDWG/H/Y	FLGITDEVTEGQFMYVTGGRLTYSNWKKDQPDDWYGYGLGGGEDCVTI	Iobst & Drickamer (1994)
QPDWG/-/G	FLGITDEVTEGQFMYVTGGRLTYSNWKKDQPDDWYGHGLGGGEDCVTI	Iobst & Drickamer (1994)
QPDF	FLGITDEVTEGQFMYVTGGRLTYSNWKKDQPDDFGSGEDCVTI	Iobst & Drickamer (1994)
QPDFG	FLGITDEVTEGQFMYVTGGRLTYSNWKKDQPDDFYGHGLGGGEDCVTI	Iobst & Drickamer (1994)
REGION 1	FLGIRKVNNVFMYVTGGRLTYSNWKKDEPNDHGSGEDCVTI	Blanck et al. (1996)
REGION 2	FLGITDEVTEGQFMYVTGGRLTYSNWKKDEPNNRQKDEDCVTI	Blanck et al. (1996)
RES. 189	FLGITDEVTEGQFMYVTGGRLTYSNWKKDEPNDGGSGEDCVTI	Torgersen et al. (1998)
RES. 197	FLGITDEVTEGQFMYVTGGRLTYSNWKKDEPNDHGSGEDCVEI	Torgersen et al. (1998)
LOOP 3E	FLGITDEVTEGQFMYVTGGRLTYSNWAPGEPNDHGSGEDCVTI	Torgersen et al. (1998)
LOOP 3P	FLGITDEVTEGQFMYVTGGRLTYSNWADNEPNDHGSGEDCVTI	Torgersen et al. (1998)
REGION 4	FLGITDEVTEGQFMYVTGGRLTYSNWKKDQPDDWYGHGLGGGEDCVHI	Kolatkar et al. (1998)
REGION 4'	FLGITDEVTEGQFMYVTGGRLTYSNWRPGQPDDWYGHGLGGGEDCVHI	Kolatkar et al. (1998)
QPDWG/QNG	FLGITDQNGQFMYVTGGRLTYSNWKKDQPDDWYGHGLGGGEDCVTI	Wragg & Drickamer (1999)
QPDWG/QNGP	FLGITDQNGPFMYVTGGRLTYSNWKKDQPDDWYGHGLGGGEDCVTI	Wragg & Drickamer (1999)
MBP/CHL189	FLGITDEVTEGQFMYVTGGRLTYSNWKEGEPNNRGSGEDCVTI	Burrows et al. (1997)
MBP/CHL192	FLGITDEVTEGQFMYVTGGRLTYSNWKEGEPNNRGFNEDCVTI	Burrows et al. (1997)
MBP/CHL208	FLGITDEVTEGQFMYVTGGRLTYSNWKEGEPNNRGFNEDCAHV	Burrows et al. (1997)
rSP-A E195Q,R197D	YLGMIEDQTPGDFHYLDGASVNYTNWYPGQPDGQGKEKCVEM	McCormack et al. (1994)
AM2	YLGMIEDQTPGDFHYLDGASVNYTNWYPGEPRGQGKEKCVTI	Honma et al. (1997)
AM3	YLGMIEDQTPGDFHYLDGASVNYTNWYPGEPNDHGSGEDCVTI	Honma et al. (1997)
E195A	YLGMIEDQTPGDFHYLDGASVNYTNWYPGAPRGQGKEKCVEM	McCormack et al. (1997)
R197G	YLGMIEDQTPGDFHYLDGASVNYTNWYPGEPGGQGKEKCVEM	McCormack et al. (1997)
E202A	YLGMIEDQTPGDFHYLDGASVNYTNWYPGEPRGQGKAKCVEM	McCormack et al. (1997)
N187S	YLGMIEDQTPGDFHYLDGASVSYTNWYPGEPRGQGKEKCVEM	McCormack et al. (1997)
R197A	YLGMIEDQTPGDFHYLDGASVNYTNWYPGEPAGQGKEKCVEM	Pattanajitvilai et al. (1998)
R197K	YLGMIEDQTPGDFHYLDGASVNYTNWYPGEPKGQGKEKCVEM	Pattanajitvilai et al. (1998)
R197H	YLGMIEDQTPGDFHYLDGASVNYTNWYPGEPHGQGKEKCVEM	Pattanajitvilai et al. (1998)

	R197D	YLGMIEDQTPGDFHYLDGASVNYTNWYPGEPDGQGKEKCVEM	Pattanajitvilai et al. (1998)
	R197N	YLGMIEDQTPGDFHYLDGASVNYTNWYPGEPNGQGKEKCVEM	Pattanajitvilai et al. (1998)
	E195Q	YLGMIEDQTPGDFHYLDGASVNYTNWYPGQPRGQGKEKCVEM	Tsunezawa et al. (1998)
	K201A	YLGMIEDQTPGDFHYLDGASVNYTNWYPGEPRGQGAEKCVEM	Tsunezawa et al. (1998)
	K203A	YLGMIEDQTPGDFHYLDGASVNYTNWYPGEPRGQGKEACVEM	Tsunezawa et al. (1998)
	E197A,K201A,K203A	YLGMIEDQTPGDFHYLDGASVNYTNWYPGAPRGQGAEACVEM	Tsunezawa et al. (1998)
	ad3	YLGMIEDQTPGDFHYLDGASVNYTNWYPGEPNNNGGAENCVEI	Sano et al. (1998)
	ad4	YLGMIEDQTEGKFTYPTGEALVYSNWAPGEPNNNGGAENCVEI	Sano et al. (1998)
	rat ama4	YLGMIEDQTEGQFMYVTGGRLTYSNWKKDEPRGQGKEKCVEM	Chiba et al (1999)
hSP-A	R199A	YVGLTEGPSPGDFRYSDGTPVNYTNWYRGEPAGAGKEQCVEM	Tsunezawa et al. (1998)
	K201A	YVGLTEGPSPGDFRYSDGTPVNYTNWYRGEPAGRGAEQCVEM	Tsunezawa et al. (1998)
	hum ama4	YVGLTEGPTEGQFMYVTGGRLTYSNWKKDEPRGRGKEQCVEM	Chiba et al (1999)
rSP-D	E321Q,N323D	FLSMTDVGTEGKFTYPTGEALVYSNWAPGQPDNNGGAENCVEI	Ogasawara & Voelker (1995)
h-esl	K67A	WIGIRKVNNVWVWVGTQAPLTEEAKNWAPGEPNNRQKDEDCVEI	Erbe et al.
	K74A	WIGIRKVNNVWVWVGTQKPLTEEAANWAPGEPNNRQKDEDCVEI	Erbe et al.
	R84A,K86A	WIGIRKVNNVWVWVGTQKPLTEEAKNWAPGEPNNAQADEDCVEI	Erbe et al.
	R84A	WIGIRKVNNVWVWVGTQKPLTEEAKNWAPGEPNNAQKDEDCVEI	Kogan et al. (1995)
	R84K	WIGIRKVNNVWVWVGTQKPLTEEAKNWAPGELNNKQKDEDCVEI	Kogan et al. (1995)
	R84K,D89G	WIGIRKVNNVWVWVGTQKPLTEEAKNWAPGEPNNKQKDEGCVEI	Kogan et al. (1995)
	A77K	WIGIRKVNNVWVWVGTQKPLTEEAKNWKPGEPNNRQKDEDCVEI	Kogan et al. (1995)
	A77K,P78K	WIGIRKVNNVWVWVGTQKPLTEEAKNWKKGEPNNRQKDEDCVEI	Kogan et al. (1995)
	A77K,P78K,R84A	WIGIRKVNNVWVWVGTQKPLTEEAKNWKKGEPNNAQKDEDCVEI	Kogan et al. (1995)
	D87E	WIGIRKVNNVWVWVGTQKPLTEEAKNWAPGEPNNRQKEEDCVEI	Kogan et al. (1995)
	D87N	WIGIRKVNNVWVWVGTQKPLTEEAKNWAPGEPNNRQKNEDCVEI	Kogan et al. (1995)
	D89N	WIGIRKVNNVWVWVGTQKPLTEEAKNWAPGEPNNRQKDENCVEI	Kogan et al. (1995)
	D89E	WIGIRKVNNVWVWVGTQKPLTEEAKNWAPGEPNNRQKDEECVEI	Kogan et al. (1995)
	A77K,E80Q,N82D	WIGIRKVNNVWVWVGTQKPLTEEAKNWKPGQPDNRQKDEDCVEI	Kogan et al. (1995)
h-psl	A77K	WIGIRKNNNKTWTWVGTKKALTNEAENWKDNEPNNKRNNEDCVEI	Revelle et al. (1996)
	A77K,E80D,N82D	WIGIRKNNNKTWTWVGTKKALTNEAENWKDNQPDNKRNNEDCVEI	Revelle et al. (1996)
MGR	2A/R	WIGLTDQNGPWRWVDGTDYEKGFTHWRPKQPDNWYGHGLGGGEDCAHF	Iobst & Drickamer (1996)
	2K/G	WIGLTDQNGPWRWVDGTDYEKGFTHWAPGQPDNWYGHGLGGGEDCAHP	Iobst & Drickamer (1996)
	2A/R,2K/G	WIGLTDQNGPWRWVDGTDYEKGFTHWRPGQPDNWYGHGLGGGEDCAHF	Iobst & Drickamer (1996)

4E/I	WIGL T D Q N G P W R W V D G T D Y E K G F T H W A P K Q P D N W Y G H G L G G G E D CAHI	Iobst & Drickamer (1996)
4H/A	WIGL T D Q N G P W R W V D G T D Y E K G F T H W A P K Q P D N W Y G H G L G G G E D CAAF	Iobst & Drickamer (1996)
4H/E	WIGL T D Q N G P W R W V D G T D Y E K G F T H W A P K Q P D N W Y G H G L G G G E D CAEF	Iobst & Drickamer (1996)
4H/Q	WIGL T D Q N G P W R W V D G T D Y E K G F T H W A P K Q P D N W Y G H G L G G G E D CAQF	Iobst & Drickamer (1996)
4H/N	WIGL T D Q N G P W R W V D G T D Y E K G F T H W A P K Q P D N W Y G H G L G G G E D CANF	Iobst & Drickamer (1996)
4H/Y	WIGL T D Q N G P W R W V D G T D Y E K G F T H W A P K Q P D N W Y G H G L G G G E D CAYF	Iobst & Drickamer (1996)
4H/D	WIGL T D Q N G P W R W V D G T D Y E K G F T H W A P K Q P D N W Y G H G L G G G E D CADF	Iobst & Drickamer (1996)
4H/K	WIGL T D Q N G P W R W V D G T D Y E K G F T H W A P K Q P D N W Y G H G L G G G E D CAKF	Iobst & Drickamer (1996)
2A/R,2K/G,4H/A	WIGL T D Q N G P W R W V D G T D Y E K G F T H W R P G Q P D N W Y G H G L G G G E D CAAF	Iobst & Drickamer (1996)
RHL 4H/A	WIGL T D Q N G P W K W V D G T D Y E T G F K N W R P G Q P D D W Y G H G L G G G E D CAAF	Iobst & Drickamer (1996)
CHL R173A	W I G L T D E N Q E G E W Q W V D G T D T R S S F T F W K E G E P N N A G F N E D C A H V	Burrows et al. (1997)
G174A	W I G L T D E N Q E G E W Q W V D G T D T R S S F T F W K E G E P N N R A F N E D C A H V	Burrows et al. (1997)
F175A	W I G L T D E N Q E G E W Q W V D G T D T R S S F T F W K E G E P N N R G A N E D C A H V	Burrows et al. (1997)
N176A	W I G L T D E N Q E G E W Q W V D G T D T R S S F T F W K E G E P N N R G F A E D C A H V	Burrows et al. (1997)

Table 2B: LSB derivatives (β3 and β4 consensus elements are underlined)

CTLD	Mut.	LSB sequence (one letter code)	Reference
hTN	TNK163A	C A V L S G A A N G A W F D K R C	Graversen et al. (1998)
	TNK166A	C A V L S G A A N G K W F D A R C	Graversen et al. (1998)
	TNR167A	C A V L S G A A N G K W F D K A C	Graversen et al. (1998)
	TNF164L	C A V L S G A A N G K W L D K R C	Graversen et al. (1998)
	TND165A	C A V L S G A A N G K W F A K R C	Graversen et al. (1998)
	TND165E	C A V L S G A A N G K W F E K R C	Graversen et al. (2000)
	TND165N	C A V L S G A A N G K W F N K R C	Graversen et al. (2000)
rMBP	I207V	C V T I V D N G L W N D V S C	Iobst et al. (1994)
	I207L	C V T I V D N G L W N D L S C	Iobst et al. (1994)
	I207A	C V T I V D N G L W N D A S C	Iobst et al. (1994)
	I207E	C V T I V D N G L W N D E S C	Torgensen et al. (1996)
	Region 4E	C V T I V Y I K R E K D N G L W N D I S C	Torgensen et al. (1996)
	Region 4P	C V T I V Y I K S P S D N G L W N D I S C	Torgensen et al. (1996)
	207VY	C V T I V D N G L W N D V Y C	Burrows et al. (1997)
	β34	C A H V W T S G Q W N D V Y C	Burrows et al. (1997)
h-esl	Y94F	C V E I F I K R E K D V G M W N D E R C	Kogan et al. (1995)
	Y94R	C V E I R I K R E K D V G M W N D E R C	Kogan et al. (1995)
	Y94D	C V E I D I K R E K D V G M W N D E R C	Kogan et al. (1995)
	Y94A	C V E I A I K R E K D V G M W N D E R C	Kogan et al. (1995)
	Y94S	C V E I S I K R E K D V G M W N D E R C	Kogan et al. (1995)
	E107D	C V E I Y I K R E K D V G M W N D D R C	Kogan et al. (1995)
	E107A	C V E I Y I K R E K D V G M W N D A R C	Kogan et al. (1995)
	E107N	C V E I Y I K R E K D V G M W N D N R C	Kogan et al. (1995)
	E107K	C V E I Y I K R E K D V G M W N D K R C	Kogan et al. (1995)
	E107Q	C V E I Y I K R E K D V G M W N D Q R C	Kogan et al. (1995)
	R97D	C V E I Y I K D E K D V G M W N D E R C	Revelle et al. (1996)
	R97S	C V E I Y I K S E K D V G M W N D E R C	Revelle et al. (1996)

	R97E	C V E I Y I K E E K D V G M W N D E R C	Revelle et al. (1996)
h-psl	K96Q	C V E I Y I Q S P S A P G M W N D E H C	Revelle et al. (1996)
	K96R	C V E I Y I R S P S A P G M W N D E H C	Revelle et al. (1996)
	K96E	C V E I Y I E S P S A P G M W N D E H C	Revelle et al. (1996)
	S97A	C V E I Y I K A P S A P G M W N D E H C	Revelle et al. (1996)
	S97D	C V E I Y I K D P S A P G M W N D E H C	Revelle et al. (1996)
	S97R	C V E I Y I K R P S A P G M W N D E H C	Revelle et al. (1996)
	REK	C V E I Y I K R E K A P G M W N D E H C	Revelle et al. (1996)
	S99D	C V E I Y I K S P D A P G M W N D E H C	Revelle et al. (1996)
CHL	V191A	C A H V W T S G Q W N D A Y C	Burrows et al. (1997)
	Y192A	C A H V W T S G Q W N D V A C	Burrows et al. (1997)

2C: Other TN CTLD derivatives

CTLD	Mut.	TN sequence (one letter code)	Reference
hTN	TNR169A	S G A A N G K W F D K R C A D Q	Graversen et al. (1998)
	TNS85G	C I S R G G T L G T P Q T	Jaquinod et al. (1999)

Notes:
- hTN: human tetranectin;
- rMBP: rat manhose binding protein,
- rSP-A: rat surfactant protein-A,
- hSP-A: human surfactant protein-A,
- rSP-D: rat surfactant protein-D;
- h-esl: human e-selectin;
- h-psl: human p-selectin;
- MGR: macrophage galactose receptor;
- RHL: rat hepatic lectin,
- CHL: chicken hepatic lectin

Normally the model CTLD is defined by having a 3D structure that conforms to the secondary-structure arrangement illustrated in Fig. 1 characterized by the following main secondary structure elements:

five β-strands and two α-helices sequentially appearing in the order β1, α1, α2, β2, β3, β4, and β5, the β-strands being arranged in two anti-parallel β-sheets, one composed of β1 and β5, the other composed of β2, β3 and β4,

at least two disulfide bridges, one connecting α1 and β5 and one connecting β3 and the polypeptide segment connecting β4 and β5,

a loop region consisting of two polypeptide segments, loop segment A (LSA) connecting β2 and β3 and comprising typically 15-70 or, less typically, 5-14 amino acid residues, and loop segment B (LSB) connecting β3 and β4 and comprising typically 5-12 or less typically, 2-4 amino acid residues.

However, also a CTLD, for which no precise 3D structure is available, can be used as a model CTLD, such CTLD being defined by showing sequence similarity to a previously recognised member of the CTLD family as expressed by an amino acid sequence identity of at least 22 %, preferably at least 25 % and more preferably at least 30 %, and by containing the cysteine residues necessary for establishing the conserved two-disulfide bridge topology (i.e. Cys_I, Cys_{II}, Cys_{III} and Cys_{IV}). The loop region, consisting of the loop segments LSA and LSB, and its flanking β-strand structural elements can then be identified by inspection of the sequence alignment with the collection of CTLDs shown in Fig. 1, which provides identification of the sequence locations of the β2- and β3-strands

with the further corroboration provided by comparison of these sequences with the four-residue consensus sequences, β2cseq and β3cseq, and the β4 strand segment located typically at positions -6 to -2 and less typically at positions -5 to -2 relative to the conserved Cys_{III} residue and with the characteristic residues at positions -5 and -3 as elucidated from Table 1 and deducted above under BACKGROUND OF THE INVENTION.

The same considerations apply for determining whether in a model CTLD the α-helices and β-strands and connecting segments are conserved to such a degree that the scaffold structure of the CTLD is substantially maintained.

It may be desirable that up to 10, preferably up to 4, and more preferably 1 or 2, amino acid residues are substituted, deleted or inserted in the α-helices and/or β-strands and/or connecting segments of the model CTLD. In particular, changes of up to 4 residues may be made in the β-strands of the model CTLD as a consequence of the introduction of recognition sites for one or more restriction endonucleases in the nucleotide sequence encoding the CTLD to facilitate the excision of part or all of the loop region and the insertion of an altered amino acid sequence instead while the scaffold structure of the CTLD is substantially maintained.

Of particular interest are proteins wherein the model CTLD is that of a tetranectin. Well known tetranectins the CTLDs of which can be used as model CTLDs are human tetranectin and murine tetranectin. The proteins according to the invention thus comprise variants of such model CTLDs.

The proteins according to the invention may comprise N-terminal and/or C-terminal extensions of the CTLD vari-

ant, and such extensions may for example contain effector, enzyme, further binding and/or multimerising functions. In particular, said extension may be the non-CTLD-portions of a native C-type lectin-like protein or C-type lectin or a "soluble" variant thereof lacking a functional transmembrane domain.

The proteins according to the invention may also be multimers of a moiety comprising the CTLD variant, e.g. derivatives of the native tetranectin trimer.

In a preferred aspect the present invention provides a combinatorial library of proteins having the scaffold structure of C-type lectin-like domains (CTLD), said proteins comprising variants of a model CTLD wherein the α-helices and β-strands are conserved to such a degree that the scaffold structure of the CTLD is substantially maintained, while the loop region or parts of the loop region of the CTLD is randomised with respect to amino acid sequence and/or number of amino acid residues.

The proteins making up such a library comprise variants of model CTLDs defined as for the above proteins according to the invention, and the variants may include the changes stated for those proteins.

In particular, the combinatorial library according to the invention may consist of proteins wherein the model CTLD is that of a tetranectin, e.g. that of human tetranectin or that of murine tetranectin.

The combinatorial library according to the invention may consist of proteins comprising N-terminal and/or C-terminal extensions of the CTLD variant, and such extensions may for example contain effector, enzyme, further binding and/or multimerising functions. In particular,

said extensions may be the non-CTLD-portions of a native C-type lectin-like protein or C-type lectin or a "soluble" variant thereof lacking a functional transmembrane domain.

The combinatorial library according to the invention may also consist of proteins that are multimers of a moiety comprising the CTLD variant, e.g. derivatives of the native tetranectin trimer.

The present invention also provides derivatives of a native tetranectin wherein up to 10, preferably up to 4, and more preferably 1 or 2, amino acid residues are substituted, deleted or inserted in the α-helices and/or β-strands and/or connecting segments of its CTLD as well as nucleic acids encoding such derivatives. Specific derivatives appear from SEQ ID Nos: 02, 04, 09, 11, 13, 15, 29, 31, 36, and 38; and nucleic acids comprising nucleotide inserts encoding specific tetranectin derivatives appear from SEQ ID Nos: 12, 14, 35, and 37.

The invention comprises a method of constructing a tetranectin derivative adapted for the preparation of a combinatorial library according to the invention, wherein the nucleic acid encoding the tetranectin derivative has been modified to generate endonuclease restriction sites within nucleic acid segments encoding β2, β3 or β4, or up to 30 nucleotides upstream or downstream in the sequence from any nucleotide which belongs to a nucleic acid segment encoding β2, β3 or β4.

The invention also comprises the use of a nucleotide sequence encoding a tetranectin, or a derivative thereof wherein the scaffold structure of its CTLD is substantially maintained, for preparing a library of nucleotide sequences encoding related proteins by randomising part

or all of the nucleic acid sequence encoding the loop region of its CTLD.

Further, the present invention provides nucleic acid comprising any nucleotide sequence encoding a protein according to the invention.

In particular, the invention provides a library of nucleic acids encoding proteins of a combinatorial library according to the invention, in which the members of the ensemble of nucleic acids, that collectively constitute said library of nucleic acids, are able to be expressed in a display system, which provides for a logical, physical or chemical link between entities displaying phenotypes representing properties of the displayed expression products and their corresponding genotypes.

In such a library the display system may be selected from

(I) a phage display system such as

(1) a filamentous phage fd in which the library of nucleic acids is inserted into

(a) a phagemid vector,

(b) the viral genome of a phage

(c) purified viral nucleic acid in purified single- or double-stranded form, or

(2) a phage lambda in which the library is inserted into

(a) purified phage lambda DNA, or

(b) the nucleic acid in lambda phage particles; or

(II) a viral display system in which the library of nucleic acids is inserted into the viral nucleic acid of a eukaryotic virus such as baculovirus; or

(III) a cell-based display system in which the library of nucleic acids is inserted into, or adjoined to, a nucleic acid carrier able to integrate either into the host genome or into an extrachromosomal element able to maintain and express itself within the cell and suitable for cell-surface display on the surface of

(a) bacterial cells,

(b) yeast cells, or

(c) mammalian cells; or

(IV) a nucleic acid entity suitable for ribosome linked display into which the library of nucleic acid is inserted; or

(V) a plasmid suitable for plasmid linked display into which the library of nucleic acid is inserted.

A well-known and useful display system is the "Recombinant Phage Antibody System" with the phagemid vector "pCANTAB 5E" supplied by Amersham Pharmacia Biotech (code no. 27-9401-01).

Further, the present invention provides a method of preparing a protein according to the invention, wherein the protein comprises at least one or more, identical or not identical, CTLD domains with novel loop-region sequences which has (have) been isolated from one or more CTLD libraries by screening or selection. At least one such CTLD domain may have been further modified by mutagenesis; and the protein containing at least one CTLD domain may have been assembled from two or more components by chemical or enzymatic coupling or crosslinking.

Also, the present invention provides a method of preparing a combinatorial library according to the invention comprising the following steps:

1) inserting nucleic acid encoding a protein comprising a model CTLD into a suitable vector,
2) if necessary, introducing restriction endonuclease recognition sites by site directed mutagenesis, said recognition sites being properly located in the sequence at or close to the ends of the sequence encoding the loop region of the CTLD or part thereof,
3) excising the DNA fragment encoding the loop region or part thereof by use of the proper restriction endonucleases,
4) ligating mixtures of DNA fragments into the restricted vector, and
5) inducing the vector to express randomised proteins having the scaffold structure of CTLDs in a suitable medium.

In a further aspect, the present invention provides a method of screening a combinatorial library according to the invention for binding to a specific target which comprises the following steps:

1) expressing a nucleic acids library according to any one of claims 59-61 to display the library of proteins in the display system;
2) contacting the collection of entities displayed with a suitably tagged target substance for which isolation of a CTLD-derived exhibiting affinity for said target substance is desired;
3) harvesting subpopulations of the entities displayed that exhibit affinity for said target substance by means of affinity-based selective extractions, utilizing the tag to which said target substance is conjugated or physically attached or adhering to as a vehicle or means of affinity purification, a procedure commonly referred to in the field as "affin-

ity panning", followed by re-amplification of the sub-library;

4) isolating progressively better binders by repeated rounds of panning and re-amplification until a suitably small number of good candidate binders is obtained; and,

5) if desired, isolating each of the good candidates as an individual clone and subjecting it to ordinary functional and structural characterisation in preparation for final selection of one or more preferred product clones.

In a still further aspect, the present invention provides a method of reformatting a protein according to the invention or selected from a combinatorial library according to the invention and containing a CTLD variant exhibiting desired binding properties, in a desired alternative species-compatible framework by excising the nucleic acid fragment encoding the loop region-substituting polypeptide and any required single framework mutations from the nucleic acid encoding said protein using PCR technology, site directed mutagenesis or restriction enzyme digestion and inserting said nucleic acid fragment into the appropriate location(s) in a display- or protein expression vector that harbours a nucleic acid sequence encoding the desired alternative CTLD framework.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows an alignment of the amino acid sequences of ten CTLDs of known 3D-structure. The sequence locations of main secondary structure elements are indicated above each sequence, labelled in sequential numerical order as "αN", denoting α-helix number N, and "βM", denoting β-strand number M.

The four cysteine residues involved in the formation of the two conserved disulfide bridges of CTLDs are indicated and enumerated in the Figure as "C_I", "C_{II}", "C_{III}" and "C_{IV}", respectively. The two conserved disulfide bridges are C_I-C_{IV} and C_{II}-C_{III}, respectively.

The ten C-type lectins are

hTN: human tetranectin [Nielsen et al. (1997)];

MBP: mannose binding protein [Weis et al. (1991); Sheriff et al. (1994)];

SP-D: surfactant protein D [Håkansson et al. (1999)];

LY49A: NK receptor LY49A [Tormo et al. (1999)];

H1-ASR: H1 subunit of the asialoglycoprotein receptor [Meier et al. (2000)];

MMR-4: macrophage mannose receptor domain 4 [Feinberg et al. (2000)];

IX-A and IX-B: coagulation factors IX/X-binding protein domain A and B, respectively [Mizuno et al. (1997)];

Lit: lithostatine [Bertrand et al. (1996)];

TU14: tunicate C-type lectin [Poget et al. (1999)].

Fig. 2 shows an alignment of the nucleotide and amino acid sequences of the coding regions of the mature forms of human and murine tetranectin with an indication of known secondary structural elements.

hTN: human tetranectin; nucleotide sequence from Berglund and Petersen (1992).

mTN: murine tetranectin; nucleotide sequence from Sørensen et al. (1995).

Secondary structure elements from Nielsen et al. (1997). "α" denotes an α-helix; "β" denotes a β-strand; and "L" denotes a loop.

Fig. 3 shows an alignment of the nucleotide and amino acid sequences of human and murine tlec coding regions. htlec: the sequence derived from hTN; mtlec: the sequence derived from mTN. The position of the restriction endonuclease sites for *Bgl* II, *Kpn* I, and *Mun* I are indicated.

Fig. 4 shows an alignment of the nucleotide and amino acid sequences of human and murine tCTLD coding regions. htCTLD: the sequence derived from hTN; mtCTLD: the sequence derived from mTN. The position of the restriction endonuclease sites for *Bgl* II, *Kpn* I, and *Mun* I are indicated.

Fig. 5 shows an outline of the pT7H6FX-htlec expression plasmid. The FX-htlec fragment was inserted into pT7H6 [Christensen et al. (1991)] between the Bam HI and Hind III cloning sites.

Fig. 6 shows the amino acid sequence (one letter code) of the FX-htlec part of the H6FX-htlec fusion protein produced by pT7H6FX-htlec.

Fig. 7 shows an outline of the pT7H6FX-htCTLD expression plasmid. The FX-htCTLD fragment was inserted into pT7H6 [Christensen et al. (1991)] between the Bam HI and Hind III cloning sites.

Fig. 8 shows the amino acid sequence (one letter code) of the FX-htCTLD part of the H6FX-htCTLD fusion protein produced by pT7H6FX-htCTLD.

Fig. 9 shows an outline of the pPhTN phagemid. The PhTN fragment was inserted into the phagemid pCANTAB 5E (Amersham Pharmacia Biotech, code no. 27-9401-01) between the Sfi I and Not I restriction sites.

Fig. 10 shows the amino acid sequence (one letter code) of the PhTN part of the PhTN-gene III fusion protein produced by pPhTN.

Fig. 11 shows an outline of the pPhTN3 phagemid. The PhTN3 fragment was inserted into the phagemid pCANTAB 5E (Amersham Pharmacia Biotech, code no. 27-9401-01) between the Sfi I and Not I restriction sites.

Fig. 12 shows the amino acid sequence (one letter code) of the PhTN3 part of the PhTN3-gene III fusion protein produced by pPhTN3.

Fig. 13 shows an outline of the pPhtlec phagemid. The Phtlec fragment was inserted into the phagemid pCANTAB 5E (Amersham Pharmacia Biotech, code no. 27-9401-01) between the Sfi I and Not I restriction sites.

Fig. 14 shows the amino acid sequence (one letter code) of the Phtlec part of the Phtlec-gene III fusion protein produced by pPhtlec.

Fig. 15 shows an outline of the pPhtCTLD phagemid. The PhtCTLD fragment was inserted into the phagemid pCANTAB 5E (Amersham Pharmacia Biotech, code no. 27-9401-01) between the Sfi I and Not I restriction sites.

Fig. 16 shows the amino acid sequence (one letter code) of the PhtCTLD part of the PhtCTLD-gene III fusion protein produced by pPhtCTLD.

Fig. 17 shows an outline of the pUC-mtlec.

Fig. 18 shows an outline of the pT7H6FX-mtlec expression plasmid. The FX-mtlec fragment was inserted into pT7H6 [Christensen et al. (1991)] between the Bam HI and Hind III cloning sites.

Fig. 19 shows the amino acid sequence (one letter code) of the FX-mtlec part of the H6FX-mtlec fusion protein produced by pT7H6FX-mtlec.

Fig. 20 shows an outline of the pT7H6FX-mtCTLD expression plasmid. The FX-mtCTLD fragment was inserted into pT7H6 [Christensen et al. (1991)] between the Bam HI and Hind III cloning sites.

Fig. 21 shows the amino acid sequence (one letter code) of the FX-mtCTLD part of the H6FX-mtCTLD fusion protein produced by pT7H6FX-mtCTLD.

Fig. 22 shows an outline of the pPmtlec phagemid. The Pmtlec fragment was inserted into the phagemid pCANTAB 5E (Amersham Pharmacia Biotech, code no. 27-9401-01) between the Sfi I and Not I restriction sites.

Fig. 23 shows the amino acid sequence (one letter code) of the Pmtlec part of the Pmtlec-gene III fusion protein produced by pPmtlec.

Fig. 24 shows an outline of the pPmtCTLD phagemid. The PmtCTLD fragment was inserted into the phagemid pCANTAB 5E (Amersham Pharmacia Biotech, code no. 27-9401-01) between the Sfi I and Not I restriction sites.

Fig. 25 shows the amino acid sequence (one letter code) of the PmtCTLD part of the PmtCTLD-gene III fusion protein produced by pPmtCTLD.

Fig. 26 shows an ELISA-type analysis of Phtlec-, PhTN3-, and M13KO7 helper phage binding to anti-tetranectin or BSA. Panel A: Analysis with 3% skimmed milk/5 mM EDTA as blocking reagent. Panel B: Analysis with 3% skimmed milk as blocking reagent.

Fig. 27 shows an ELISA-type analysis of Phtlec-, PhTN3-, and M13KO7 helper phage binding to plasminogen (Plg) and BSA. Panel A: Analysis with 3% skimmed milk/5 mM EDTA as blocking reagent. Panel B: Analysis with 3% skimmed milk as blocking reagent.

Fig. 28 shows an ELISA-type analysis of the B series and C series polyclonal populations, from selection round 2, binding to plasminogen (Plg) compared to background.

Fig. 29 Phages from twelve clones isolated from the third round of selection analysed for binding to hen egg white lysozyme, human β_2-microglobulin and background in an ELISA-type assay.

Fig. 30 shows the amino acid sequence (one letter code) of the PrMBP part of the PrMBP-gene III fusion protein produced by pPrMBP.

Fig. 31 shows an outline of the pPrMBP phagemid. The PrMBP fragment was inserted into the phagemid pCANTAB 5E (Amersham Pharmacia Biotech, code no. 27-9401-01) between the Sfi I and Not I restriction sites.

Fig. 32 shows the amino acid sequence (one letter code) of the PhSP-D part of the PhSP-D-gene III fusion protein produced by pPhSP-D.

Fig. 33 shows an outline of the pPhSP-D phagemid. The PhSP-D fragment was inserted into the phagemid pCANTAB 5E

(Amersham Pharmacia Biotech, code no. 27-9401-01) between the Sfi I and Not I restriction sites.

Fig. 34. Phages from 48 clones isolated from the third round of selection in the #1 series analysed for binding to hen egg white lysozyme and to A-HA in an ELISA-type assay.

Fig. 35. Phages from 48 clones isolated from the third round of selection in the #4 series analysed for binding to hen egg white lysozyme and to A-HA in an ELISA-type assay.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

The terms "C-type lectin-like protein" and "C-type lectin" are used to refer to any protein present in, or encoded in the genomes of, any eukaryotic species, which protein contains one or more CTLDs or one or more domains belonging to a subgroup of CTLDs, the CRDs, which bind carbohydrate ligands. The definition specifically includes membrane attached C-type lectin-like proteins and C-type lectins, "soluble" C-type lectin-like proteins and C-type lectins lacking a functional transmembrane domain and variant C-type lectin-like proteins and C-type lectins in which one or more amino acid residues have been altered in vivo by glycosylation or any other post-synthetic modification, as well as any product that is obtained by chemical modification of C-type lectin-like proteins and C-type lectins.

In the claims and throughout the specification certain alterations may be defined with reference to amino acid residue numbers of a CTLD domain or a CTLD-containing

protein. The amino acid numbering starts at the first N-terminal amino acid of the CTLD or the native or artificial CTLD-containing protein product, as the case may be, which shall in each case be indicated by unambiguous external literature reference or internal reference to a figure contained herein within the textual context.

The terms "amino acid", "amino acids" and "amino acid residues" refer to all naturally occurring L-α-amino acids. This definition is meant to include norleucine, ornithine, and homocysteine. The amino acids are identified by either the single-letter or three-letter designations:

Asp	D	aspartic acid	Ile	I	isoleucine
Thr	T	threonine	Leu	L	leucine
Ser	S	serine	Tyr	Y	tyrosine
Glu	E	glutamic acid	Phe	F	phenylalanine
Pro	P	proline	His	H	histidine
Gly	G	glycine	Lys	K	lysine
Ala	A	alanine	Arg	R	arginine
Cys	C	cysteine	Trp	W	tryptophan
Val	V	valine	Gln	Q	glutamine
Met	M	methionine	Asn	N	asparagine
Nle	J	norleucine	Orn	O	ornithine
Hcy	U	homocysteine	Xxx	X	any L-α-amino acid.

The naturally occurring L-α-amino acids may be classified according to the chemical composition and properties of their side chains. They are broadly classified into two groups, charged and uncharged. Each of these groups is divided into subgroups to classify the amino acids more accurately:

A. Charged Amino Acids

Acidic Residues:	Asp, Glu
Basic Residues:	Lys, Arg, His, Orn

B. Uncharged Amino Acids

Hydrophilic Residues:	Ser, Thr, Asn, Gln
Aliphatic Residues:	Gly, Ala, Val, Leu, Ile, Nle
Non-polar Residues:	Cys, Met, Pro, Hcy
Aromatic Residues:	Phe, Tyr, Trp

The terms "amino acid alteration" and "alteration" refer to amino acid substitutions, deletions or insertions or any combinations thereof in a CTLD amino acid sequence. In the CTLD variants of the present invention such alteration is at a site or sites of a CTLD amino acid sequence. Substitutional variants herein are those that have at least one amino acid residue in a native CTLD sequence removed and a different amino acid inserted in its place at the same position. The substitutions may be single, where only one amino acid in the molecule has been substituted, or they may be multiple, where two or more amino acids have been substituted in the same molecule.

The designation of the substitution variants herein consists of a letter followed by a number followed by a letter. The first (leftmost) letter designates the amino acid in the native (unaltered) CTLD or CTLD-containing protein. The number refers to the amino acid position where the amino acid substitution is being made, and the second (righthand) letter designates the amino acid that is used to replace the native amino acid. As mentioned above, the numbering starts with "1" designating the N-terminal amino acid sequence of the CTLD or the CTLD-containing protein, as the case may be. Multiple alterations are separated by a comma (,) in the notation for ease of reading them.

The terms "nucleic acid molecule encoding", "DNA sequence encoding", and "DNA encoding" refer to the order or se-

quence of deoxyribonucleotides along a strand of deoxyribonucleic acid. The order of these deoxyribonucleotides determines the order of amino acids along the polypeptide chain. The DNA sequence thus encodes the amino acid sequence.

The terms "mutationally randomised sequence", "randomised polypeptide segment", "randomised amino acid sequence", "randomised oligonucleotide" and "mutationally randomised sequence", as well as any similar terms used in any context to refer to randomised sequences, polypeptides or nucleic acids, refer to ensembles of polypeptide or nucleic acid sequences or segments, in which the amino acid residue or nucleotide at one or more sequence positions may differ between different members of the ensemble of polypeptides or nucleic acids, such that the amino acid residue or nucleotide occurring at each such sequence position may belong to a set of amino acid residues or nucleotides that may include all possible amino acid residues or nucleotides or any restricted subset thereof. Said terms are often used to refer to ensembles in which the number of amino acid residues or nucleotides is the same for each member of the ensemble, but may also be used to refer to such ensembles in which the number of amino acid residues or nucleotides in each member of the ensemble may be any integer number within an appropriate range of integer numbers.

II. Construction and utility of combinatorial CTLD libraries

Several systems displaying phenotype, in terms of putative ligand binding modules or modules with putative enzymatic activity, have been described. These include: phage display (e.g. the filamentous phage fd [Dunn (1996), Griffiths amd Duncan (1998), Marks et al.

(1992)], phage lambda [Mikawa et al. (1996)]), display on eukarotic virus (e.g. baculovirus [Ernst et al. (2000)]), cell display (e.g. display on bacterial cells [Benhar et al. (2000)], yeast cells [Boder and Wittrup (1997)], and mammalian cells [Whitehorn et al. (1995)], ribosome linked display [Schaffitzel et al. (1999)], and plasmid linked display [Gates et al. (1996)].

The most commonly used method for phenotype display and linking this to genotype is by phage display. This is accomplished by insertion of the reading frame encoding the scaffold protein or protein of interest into an intra-domain segment of a surface exposed phage protein. The filamentous phage fd (e.g. M13) has proven most useful for this purpose. Polypeptides, protein domains, or proteins are the most frequently inserted either between the "export" signal and domain 1 of the fd gene III protein or into a so-called hinge region between domain 2 and domain 3 of the fd-phage gene III protein. Human antibodies are the most frequently used proteins for the isolation of new binding units, but other proteins and domains have also been used (e.g. human growth hormone [Bass et al. (1990)], alkaline phosphatase [McCafferty et al. (1991)], β-lactamase inhibitory protein [Huang et al. (2000)], and cytotoxic T lymphocyte-associated antigen 4 [Hufton et al. (2000)]. The antibodies are often expressed and presented as scFv or Fab fusion proteins. Three strategies have been employed. Either a specific antibody is used as a scaffold for generating a library of mutationally randomised sequences within the antigen binding clefts [e.g. Fuji et al. (1998)] or libraries representing large ensembles of human antibody encoding genes from non-immunised hosts [e.g. Nissim et al. (1994)] or from immunised hosts [e.g. Cyr and Hudspeth (2000)] are cloned into the fd phage vector.

The general procedure for accomplishing the generation of a display system for the generation of CTLD libraries comprise essentially

(1) identification of the location of the loop-region, by referring to the 3D structure of the CTLD of choice, if such information is available, or, if not, identification of the sequence locations of the β2-, β3- and β4 strands by sequence alignment with the sequences shown in Fig. 1, as aided by the further corroboration by identification of sequence elements corresponding to the β2 and β3 consensus sequence elements and β4-strand characteristics, also disclosed above;

(2) subcloning of a nucleic acid fragment encoding the CTLD of choice in a protein display vector system with or without prior insertion of endonuclease restriction sites close to the sequences encoding β2, β3 and β4; and

(3) substituting the nucleic acid fragment encoding some or all of the loop-region of the CTLD of choice with randomly selected members of an ensemble consisting of a multitude of nucleic acid fragments which after insertion into the nucleic acid context encoding the receiving framework will substitute the nucleic acid fragment encoding the original loop-region polypeptide fragments with randomly selected nucleic acid fragments. Each of the cloned nucleic acid fragments, encoding a new polypeptide replacing an original loop-segment or the entire loop-region, will be decoded in the reading frame determined within its new sequence context.

Nucleic acid fragments may be inserted in specific locations into receiving nucleic acids by any common method of molecular cloning of nucleic acids, such as by appropriately designed PCR manipulations in which chemically synthesized nucleic acids are copy-edited into the receiving nucleic acid, in which case no endonuclease restriction sites are required for insertion. Alternatively, the insertion/excision of nucleic acid fragments may be facilitated by engineering appropriate combinations of endonuclease restriction sites into the target nucleic acid into which suitably designed oligonucleotide fragments may be inserted using standard methods of molecular cloning of nucleic acids.

It will be apparent that interesting CTLD variants isolated from CTLD libraries in which restriction endonuclease sites have been inserted for convenience may contain mutated or additional amino acid residues that neither correspond to residues present in the original CTLD nor are important for maintaining the interesting new affinity of the CTLD variant. If desirable, e.g. in case the product needs to be rendered as non-immunogenic as possible, such residues may be altered or removed by back-mutation or deletion in the specific clone, as appropriate.

The ensemble consisting of a multitude of nucleic acid fragments may be obtained by ordinary methods for chemical synthesis of nucleic acids by directing the step-wise synthesis to add pre-defined combinations of pure nucleotide monomers or a mixture of any combination of nucleotide monomers at each step in the chemical synthesis of the nucleic acid fragment. In this way it is possible to generate any level of sequence degeneracy, from one unique nucleic acid sequence to the most complex mixture,

which will represent a complete or incomplete representation of maximum number unique sequences of 4^N, where N is the number of nucleotides in the sequence.

Complex ensembles consisting of multitudes of nucleic acid fragments may, alternatively, be prepared by generating mixtures of nucleic acid fragments by chemical, physical or enzymatic fragmentation of high-molecular mass nucleic acid compositions like, e.g., genomic nucleic acids extracted from any organism. To render such mixtures of nucleic acid fragments useful in the generation of molecular ensembles, as described here, the crude mixtures of fragments, obtained in the initial cleavage step, would typically be size-fractionated to obtain fragments of an approximate molecular mass range which would then typically be adjoined to a suitable pair of linker nucleic acids, designed to facilitate insertion of the linker-embedded mixtures of size-restricted oligonucleotide fragments into the receiving nucleic acid vector.

To facilitate the construction of combinatorial CTLD libraries in tetranectin, the model CTLD of the preferred embodiment of the invention, suitable restriction sites located in the vicinity of the nucleic acid sequences encoding β2, β3 and β4 in both human and murine tetranectin were designed with minimal perturbation of the polypeptide sequence encoded by the altered sequences. It was found possible to establish a design strategy, as detailed below, by which identical endonuclease restriction sites could be introduced at corresponding locations in the two sequences, allowing interesting loop-region variants to be readily excised from a recombinant murine CTLD and inserted correctly into the CTLD framework of human tetranectin or *vice versa*.

Analysis of the nucleotide sequence encoding the mature form of human tetranectin reveals (Fig. 2) that a recognition site for the restriction endonuclease *Bgl* II is found at position 326 to 331 (AGATCT), involving the encoded residues Glu109, Ile110, and Trp111 of β2, and that a recognition site for the restriction endonuclease *Kas I* is found at position 382 to 387 (GGCGCC), involving the encoded amino acid residues Gly128 and Ala129 (located C-terminally in loop 2).

Mutation, by site directed mutagenesis, of G513 to A and of C514 to T in the nucleotide sequence encoding human tetranectin would introduce a *Mun I* restriction endonuclease recognition site therein, located at position 511 to 516, and mutation of G513 to A in the nucleotide sequence encoding murine tetranectin would introduce a *Mun I* restriction endonuclease site therein at a position corresponding to the *Mun I* site in human tetranectin, without affecting the amino acid sequence of either of the encoded protomers. Mutation, by site directed mutagenesis, of C327 to G and of G386 to C in the nucleotide sequence encoding murine tetranectin would introduce a *Bgl* II and a *Kas* I restriction endonuclease recognition site, respectively, therein. Additionally, A325 in the nucleotide sequence encoding murine tetranectin is mutagenized to a G. These three mutations would affect the encoded amino acid sequence by substitution of Asn109 to Glu and Gly129 to Ala, respectively. Now, the restriction endonuclease *Kas I* is known to exhibit marked site preference and cleaves only slowly the tetranectin coding region. Therefore, a recognition site for another restriction endonuclease substituting the *Kas I* site is preferred (e.g. the recognition site for the restriction endonuclease *Kpn I*, recognition sequence GGTACC). The nucleotide and amino acid sequences of the resulting

tetranectin derivatives, human tetranectin lectin (htlec) and murine tetranectin lectin (mtlec) are shown in Fig. 3. The nucleotide sequences encoding the htlec and mtlec protomers may readily be subcloned into devices enabling protein display of the linked nucleotide sequence (e.g. phagemid vectors) and into plasmids designed for heterologous expression of protein [e.g. pT7H6, Christensen et al. (1991)]. Other derivatives encoding only the mutated CTLDs of either htlec or mtlec (htCTLD and mtCTLD, respectively) have also been constructed and subcloned into phagemid vectors and expression plasmids, and the nucleotide and amino acid sequences of these CTLD derivatives are shown in Fig. 4.

The presence of a common set of recognition sites for the restriction endonucleases *Bgl* II, *Kas* I or *Kpn* I, and *Mun* I in the ensemble of tetranectin and CTLD derivatives allows for the generation of protein libraries with randomised amino acid sequence in one or more of the loops and at single residue positions in β4 comprising the lectin ligand binding region by ligation of randomised oligonucleotides into properly restricted phagemid vectors encoding htlec, mtlec, htCTLD, or mtCTLD derivatives.

After rounds of selection on specific targets (e.g. eukaryotic cells, virus, bacteria, specific proteins, polysaccharides, other polymers, organic compounds etc.) DNA may be isolated from the specific phages, and the nucleotide sequence of the segments encoding the ligand-binding region determined, excised from the phagemid DNA and transferred to the appropriate derivative expression vector for heterologous production of the desired product. Heterologous production in a prokaryote may be preferred because an efficient protocol for the isolation and refolding of tetranectin and derivatives has been reported

(International Patent Application Publication WO 94/18227 A2).

A particular advantage gained by implementing the technology of the invention, using tetranectin as the scaffold structure, is that the structures of the murine and human tetranectin scaffolds are almost identical, allowing loop regions to be swapped freely between murine and human tetranectin derivatives with retention of functionality. Swapping of loop regions between the murine and the human framework is readily accomplished within the described system of tetranectin derivative vectors, and it is anticipated, that the system can be extended to include other species (e.g. rat, old and new world monkeys, dog, cattle, sheep, goat etc.) of relevance in medicine or veterinary medicine in view of the high level of homology between man and mouse sequences, even at the genetic level. Extension of this strategy to include more species may be rendered possible as and when tetranectin is eventually cloned and/or sequenced from such species.

Because the C-type lectin ligand-binding region represents a different topological unit compared to the antigen binding clefts of the antibodies, we envisage that the selected binding specificities will be of a different nature compared to the antibodies. Further, we envisage that the tetranectin derivatives may have advantages compared to antibodies with respect to specificity in binding sugar moieties or polysaccharides. The tetranectin derivatives may also be advantageous in selecting binding specificities against certain natural or synthetic organic compounds.

Several CTLDs are known to bind calcium ions, and binding of other ligands is often either dependent on calcium (e.g. the collectin family of C-type lectins, where the

calcium ion bound in site 2 is directly involved in binding the sugar ligand [Weis and Drickamer (1996)]) or sensitive to calcium (e.g. tetranectin, where binding of calcium involves more of the side chains known otherwise to be involved in plasminogen kringle 4 binding [Graversen et al. (1998)]). The calcium binding sites characteristic of the C-type lectin-like protein family are comprised by residues located in loop 1, loop 4 and β-strand 4 and are dependent on the presence of a proline residue (often interspacing loop 3 and loop 4 in the structure), which upon binding is found invariantly in the *cis* conformation. Moreover, binding of calcium is known to enforce structural changes in the CTLD loop-region [Ng et al. (1998a,b)]. We therefore envisage, that binding to a specific target ligand by members of combinational libraries with preserved CTLD metal binding sites may be modulated by addition or removal of divalent metal ions (e.g. calcium ions) either because the metal ion may be directly involved in binding, because it is a competitive ligand, or because binding of the metal ion enforces structural rearrangements within the putative binding site.

The trimeric nature of several members of the C-type lectin and C-type lectin-like protein family, including tetranectin, and the accompanying avidity in binding may also be exploited in the creation of binding units with very high binding affinity.

As can be appreciated from the disclosure above, the present invention has a broad general scope and a wide area of application. Accordingly, the following examples, describing various embodiments thereof, are offered by way

of illustration only, not by way of limitation.

Example 1

Construction of tetranectin derived *E.coli* expression plasmids and phagemids

The expression plasmid pT7H6FX-htlec, encoding the FX-htlec (SEQ ID NO:01) part of full length H6FX-htlec fusion protein, was constructed by a series of four consecutive site-directed mutagenesis experiments starting from the expression plasmid pT7H6-rTN 123 [Holtet et al. (1997)] using the QuickChange™ Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA) and performed as described by the manufacturer. Mismatching primer pairs introducing the desired mutations were supplied by DNA Technology (Aarhus, Denmark). An outline of the resulting pT7H6FX-htlec expression plasmid is shown in Fig. 5, and the nucleotide sequence of the FX-htlec encoding insert is given as SEQ ID NO:01. The amino acid sequence of the FX-htlec part of the H6FX-htlec fusion protein is shown in Fig. 6 and given as SEQ ID NO:02.

The expression plasmid pT7H6FX-htCTLD, encoding the FX-htCTLD (SEQ ID NO: 03) part of the H6FX-htCTLD fusion protein, was constructed by amplification and subcloning into the plasmid pT7H6 (i.e. amplification in a polymerase chain reaction using the expression plasmid pT7H6-htlec as template, and otherwise the primers, conditions, and subcloning procedure described for the construction of the expression plasmid pT7H6TN3 [Holtet et al. (1997)]. An outline of the resulting pT7H6FX-htCTLD expression plasmid is shown in Fig. 7, and the nucleotide sequence of the FX-htCTLD encoding insert is given as SEQ ID NO:03. The amino acid sequence of the FX-htCTLD part

of the H6FX-htCTLD fusion protein is shown in Fig. 8 and given as SEQ ID NO:04.

The phagemids, pPhTN and pPhTN3, were constructed by ligation of the *Sfi* I and *Not* I restricted DNA fragments amplified from the expression plasmids pT7H6-rTN 123 (with the oligonucleotide primers 5-CGGCTGAGCGGCCCA-GCCGGCCATGGCCGAGCCACCAACCCAGAAGC-3′ [SEQ ID NO:05] and 5′-CCTGCGGCCGCCACGATCCCGAACTGG-3′ [SEQ ID NO:06]) and pT7H6FX-htCTLD (with the oligonucleotide primers 5′-CGGCTGAGCGGCCCAGCCGGCCATGGCCGCCCTGCAGACGGTC-3′ [SEQ ID NO:07] and 5′-CCTGCGGCCGCCACGATCCCGAACTGG-3′ [SEQ ID NO:06]), respectively, into a *Sfi* I and *Not* I precut vector, pCANTAB 5E supplied by Amersham Pharmacia Biotech (code no. 27-9401-01) using standard procedures. Outlines of the resulting pPhTN and pPhTN3 phagemids are shown in Fig. 9 and Fig. 11, respectively, and the nucleotide sequences of the PhTN and PhTN3 inserts are given as SEQ ID NO:08 and SEQ ID NO:10, respectively. The amino acid sequences encoded by the PhTN and PhTN3 inserts are shown in Fig 10 (SEQ ID NO:09) and Fig. 12 (SEQ ID NO:11), respectively.

The phagemids, pPhtlec and pPhtCTLD, were constructed by ligation of the *Sfi* I and *Not* I restricted DNA fragments amplified from the expression plasmids pT7H6FX-htlec (with the oligonucleotide primers 5-CGGCTGAGCGGCCCAGCC-GGCCATGGCCGAGCCACCAACCCAGAAGC-3′ [SEQ ID NO:05] and 5′-CCTGCGGCCGCCACGATCCCGAACTGG-3′ [SEQ ID NO:06]) and pT7H6FX-htCTLD (with the oligonucleotide primers 5′-CGGCTGAGCGGCCCAGCCGGCCATGGCCGCCCTGCAGACGGTC-3′ [SEQ ID NO:07] and 5′-CCTGCGGCCGCCACGATCCCGAACTGG-3′ [SEQ ID NO:06]), respectively, into a *Sfi* I and *Not* I precut vector, pCANTAB 5E supplied by Amersham Pharmacia Biotech (code no. 27-9401-01) using standard procedures. Outlines

of the resulting pPhtlec and pPhtCTLD phagemids are shown in Fig. 13 and Fig. 15, respectively, and the nucleotide sequences of the Phtlec and PhtCTLD inserts are given as SEQ ID NO:12 and SEQ ID NO:14, repectively. The amino acid sequences encoded by the Phtlec and PhtCTLD inserts are shown in Fig. 14 (SEQ ID NO:13) and Fig. 16 (SEQ ID NO:15), respectively.

A plasmid clone, pUC-mtlec, containing the nucleotide sequence corresponding to the murine tetranectin derivative mtlec (Fig. 3 and SEQ ID NO:16) was constructed by four succesive subclonings of DNA subfragments in the following way: First, two oligonucleotides 5′-CGGAATTCGAGTCACCCACTCCCAAGGCCAAGAAGGCTGCAAATGCCAAGAAA-GATTTGGTGAGCTCAAAGATGTTC-3′ (SEQ ID NO:17) and 5′-GCG-GATCCAGGCCTGCTTCTCCTTCAGCAGGGCCACCTCCTGGGCCAGGACATCCAT-CCTGTTCTTGAGCTCCTCGAACATCTTTGAGCTCACC-3′ (SEQ ID NO:18) were annealed and after a filling in reaction cut with the restriction endonucleases *Eco* RI (GAATTC) and *Bam* HI (GGATCC) and ligated into *Eco* RI and *Bam* HI precut pUC18 plasmid DNA. Second, a pair of oligonucleotides 5′-GCA-GGCCTTACAGACTGTGTGCCTGAAGGGCACCAAGGTGAACTTGAAGTGCCTCCT-GGCCTTCACCCAACCGAAGACCTTCCATGAGGCGAGCGAG-3′ (SEQ ID NO:19) and 5′-CCGCATGCTTCGAACAGCGCCTCGTTCTCTAGCTCTGAC-TGCGGGGTGCCCAGCGTGCCCCCTTGCGAGATGCAGTCCTCGCTCGCCTCATGG-3′ (SEQ ID NO:20) was annealed and after a filling in reaction cut with the restriction endonucleases *Stu* I (AGGCCT) and *Sph* I (GCATGC) and ligated into the *Stu* I and *Sph* I precut plasmid resulting from the first ligation. Third, an oligonucleotide pair 5′-GGTTCGAATACGCGC-GCCACAGCGTGGGCAACGATGCGGAGATCTAAATGCTCCCAATTGC-3′ (SEQ ID NO:21) and 5′-CCAAGCTTCACAATGGCAAACTGGCAGATGTAGGGCAATTGG-GAGCATTTAGATC-3′ (SEQ ID NO: 22) was annealed and after a filling in reaction cut with the restriction endonucleases *BstB* I (TTCGAA) and *Hind* III (AAGCTT) and ligated

into the *BstB* I and *Hind* III precut plasmid resulting from the second ligation. Fourth, an oligonucleotide pair 5′-CGGAGATCTGGCTGGGCCTCAACGACATGGCCGCGGAAGGCGCCTGGGTGGA-CATGACCGGTACCCTCCTGGCCTACAAGAACTGG-3′ (SEQ ID NO:23) and 5′-GGGCAATTGATCGCGGCATCGCTTGTCGAACCTCTTGCCGTTGGCTGCGCCAG-ACAGGGCGGCGCAGTTCTCGGCTTTGCCGCCGTCGGGTTGCGTCGTGATCTCCGTC-TCCCAGTTCTTGTAGGCCAGG-3′ (SEQ ID NO:24) was annealed and after a filling in reaction cut with the restriction endonucleases *Bgl* II (AGATCT) and *Mun* I (CAATTG) and ligated into the *Bgl* II and *Mun* I precut plasmid resulting from the third ligation. An outline of the pUC-mtlec plasmid is shown in Fig. 17, and the resulting nucleotide sequence of the *Eco* RI to *Hind* III insert is given as SEQ ID NO:16.

The expression plasmids pT7H6FX-mtlec and pT7H6FX-mtCTLD may be constructed by ligation of the *Bam* HI and *Hind* III restricted DNA fragments, amplified from the pUC-mtlec plasmid with the oligonucleotide primer pair 5-CTGGGATCC-ATCCAGGGTCGCGAGTCACCCACTCCCAAGG-3′ (SEQ ID NO:25) and 5′-CCGAAGCTTACACAATGGCAAACTGGC-3′ (SEQ ID NO:26), and with the oligonucleotide primer pair 5′- CTGGGATCCATCCAGGGTCG-CGCCTTACAGACTGTGGTC-3′ (SEQ ID NO:27), and 5′-CCGAAGCTT-ACACAATGGCAAACTGGC-3′ (SEQ ID NO:26), respectively, into *Bam* HI and *Hind* III precut pT7H6 vector using standard procedures. An outline of the expression plasmids pT7H6FX-mtlec and pT7H6FX-mtCTLD is shown in Fig. 18 and Fig. 20, respectively, and the nucleotide sequences of the FX-mtlec and FX-mtCTLD inserts are given as SEQ ID NO:28 and SEQ ID NO:30, respectively. The amino acid sequences of the FX-mtlec and FX-mtCTLD parts of the fusion proteins H6FX-mtlec and H6FX-mtCTLD fusion proteins are shown in Fig. 19 (SEQ ID NO:29) and Fig. 21 (SEQ ID NO:31), respectively.

The phagemids pPmtlec and pPmtCTLD may be constructed by ligation of the Sfi I and *Not* I restricted DNA fragments (amplified from the pUC-mtlec plasmid with the oligonucleotide primer pair 5-CGGCTGAGCGGCCCAGCCGGCCATGGCCGAGTCACCCACTCCCAAGG-3′ [SEQ ID NO:32], and 5′-CCTGCGGCCGCCACGATCCCGAACTGG-3′ [SEQ ID NO:33] and with the oligonucleotide primers 5′-CGGCTGAGCGGCCCAGCCGGCCATGGCCGCCTTACAGACTGTGGTC-3′ [SEQ ID NO:34] and 5′-CCTGCGGCCGCCACGATCCCGAACTGG-3′ [SEQ ID NO:33], respectively) into a *Sfi* I and *Not* I precut vector pCANTAB 5E supplied by Amersham Pharmacia Biotech (code no. 27-9401-01) using standard procedures. Outlines of the pPmtlec and pPmtCTLD plasmids are shown in Fig. 22 and Fig. 24, respectively, and the resulting nucleotide sequences of the Pmtlec and PmtCTLD inserts are given as SEQ ID NO:35 and SEQ ID NO:37, repectively. The amino acid sequences encoded by the Pmtlec and PmtCTLD inserts are shown in Fig. 23 (SEQ ID NO: 36) and Fig. 25 (SEQ ID NO: 38), respectively.

Example 2

Demonstration of successful display of Phtlec and PhTN3 on phages.

In order to verify that the Phtlec and PhTN3 Gene III fusion proteins can indeed be displayed by the recombinant phage particles, the phagemids pPhtlec and pPhTN3 (described in Example 1) were transformed into *E. coli* TG1 cells and recombinant phages produced upon infection with the helper phage M13KO7. Recombinant phages were isolated by precipitation with poly(ethylene glycol) (PEG 8000) and samples of Phtlec and PhTN3 phage preparations as well as a sample of helper phage were subjected to an ELISA-type sandwich assay, in which wells of a Maxisorb (Nunc) multiwell plate were first incubated with anti-

human tetranectin or bovine serum albumin (BSA) and blocked in skimmed milk or skimmed milk/EDTA. Briefly, cultures of pPhtlec and pPhTN3 phagemid transformed TG1 cells were grown at 37 °C in 2xTY-medium supplemented with 2% glucose and 100 mg/L ampicillin until A_{600} reached 0.5. By then the helper phage, M13K07, was added to a concentration of $5x10^9$ pfu/mL. The cultures were incubated at 37 °C for another 30 min before cells were harvested by centrifugation and resuspended in the same culture volume of 2xTY medium supplemented with 50 mg/L kanamycin and 100 mg/L ampicillin and transferred to a fresh set of flasks and grown for 16 hours at 25 °C. Cells were removed by centrifugation and the phages precipitated from 20 mL culture supernatant by the addition of 6 mL of ice cold 20% PEG 8000, 2.5 M NaCl. After mixing the solution was left on ice for one hour and centrifuged at 4 °C to isolate the precipitated phages. Each phage pellet was resuspended in 1 mL of 10 mM tris-HCl pH 8, 1 mM EDTA (TE) and incubated for 30 min before centrifugation. The phage containing supernatant was transferred to a fresh tube. Along with the preparation of phage samples, the wells of a Maxisorb plate was coated overnight with (70 µL) rabbit anti-human tetranectin (a polyclonal antibody from DAKO A/S, code no. A0371) in a 1:2000 dilution or with (70 µL) BSA (10 mg/mL). Upon coating, the wells were washed three times with PBS (2.68 mM KCl, 1.47 mM KH_2PO_4, 137 mM NaCl, 8.10 mM Na_2HPO_4, pH 7.4) and blocked for one hour at 37 °C with 280 µL of either 3% skimmed milk in PBS, or 3% skimmed milk, 5mM EDTA in PBS. Anti-tetranectin coated and BSA coated wells were then incubated with human Phtlec-, PhTN3-, or helper phage samples for 1 hour and then washed 3 times in PBS buffer supplemented with the appropriate blocking agent. Phages in the wells were detected after incubation with HRP-conjugated anti-phage conjugate (Amersham Pharmacia,

code no. 27-9421-01) followed by further washing. HRP activities were then measured in a 96-well ELISA reader using a standard HRP chromogenic substrate assay.

Phtlec and PhTN3 phages produced strong responses (14 times background) in the assay, irrespective of the presence or absence of EDTA in the blocking agent, whereas helper phage produced no response above background readings in either blocking agent. Only low binding to BSA was observed (Fig. 26).

It can therefore be concluded that the human Phtlec and PhTN3 phages both display epitopes that are specifically recognized by the anti-human tetranectin antibody.

Example 3

Demonstration of authentic ligand binding properties of Phtlec and PhTN3 displayed on phage

The apo-form of the CTLD domain of human tetranectin binds in a lysine-sensitive manner specifically to the kringle 4 domain of human plasminogen [Graversen et al. (1998)]. Binding of tetranectin to plasminogen can be inhibited by calcium which binds to two sites in the ligand-binding site in the CTLD domain (Kd approx. 0.2 millimolar) or by lysine-analogues like AMCHA (6-aminocyclohexanoic acid), which bind specifically in the two stronger lysine-binding sites in plasminogen of which one is located in kringle 1 and one is located in kringle 4 (Kd approx. 15 micromolar).

To demonstrate specific AMCHA-sensitive binding of human Phtlec and PhTN3 phages to human plasminogen, an ELISA assay, in outline similar to that employed to demonstrate

the presence of displayed Phlec and PhCTLD GIII fusion proteins on the phage particles (cf. Example 2), was devised.

Wells were coated with solutions of human plasminogen (10 µg/mL), with or without addition of 5mM AMCHA. Control wells were coated with BSA. Two identical arrays were established, one was subjected to blocking of excess binding capacity with 3% skimmed milk, and one was blocked using 3% skimmed milk supplemented with 5mM EDTA. Where appropriate, blocking, washing and phage stock solutions were supplemented by 5mM AMCHA. The two arrays of wells were incubated with either Phtlec-, or PhTN3-, or helper phage samples, and after washing the amount of phage bound in each well was measured using the HRP-conjugated antiphage antibody as above. The results are shown in Fig. 27, panels A and B, and can be summarized as follows

(a) In the absence of AMCHA, binding of human Phtlec phages to plasminogen-coated wells generated responses at 8-10 times background levels using either formulation of blocking agent, whereas human PhTN3 phages generated responses at 4 (absence of EDTA) or 7 (presence of EDTA) times background response levels.

(b) In the presence of 5mM AMCHA, binding of human Phtlec- and PhTN3 phages to plasminogen was found to be completely abolished.

(c) Phtlec and PhTN3 phages showed no binding to BSA, and control helper phages showed no binding to any of the immobilized substances.

(d) Specific binding of human Phtlec and PhTN3 phages to a specific ligand at moderate binding strength

(about 20 micromolar level) can be detected with high efficiency at virtually no background using a skimmed-milk blocking agent, well-known in the art of combinatorial phage technology as a preferred agent effecting the reduction of non-specific binding.

In conclusion, the results show that the Phtlec and PhTN3 Gene III fusion proteins displayed on the phage particles exhibit plasminogen-binding properties corresponding to those of authentic tetranectin, and that the physical and biochemical properties of Phtlec and PhTN3 phages are compatible with their proposed use as vehicles for the generation of combinatorial libraries from which CTLD derived units with new binding properties can be selected.

Example 4

Construction of the phage libraries Phtlec-1b001 and Phtlec-1b002.

All oligonucleotides used in this example were supplied by DNA Technology (Aarhus, Denmark).

The phage library Phtlec-1b001, containing random amino acid residues corresponding to Phtlec (SEQ ID NO: 12) positions 141-146 (loop 3), 150-153 (part of loop 4), and residue 168 (Phe in β4), was constructed by ligation of 20 µg *Kpn*I and *Mun*I restricted pPhtlec phagemid DNA (cf, Example 1) with 10 µg of *Kpn*I and *Mun*I restricted DNA fragment amplified from the oligonucleotide htlec-lib1-tp (SEQ ID NO: 39), where N denotes a mixture of 25% of each of the nucleotides T, C, G, and A, respectively and S denotes a mixture of 50 % of C and G, encoding the appropriately randomized nucleotide sequence and the oligonu-

cleotides htlec-lib1-rev (SEQ ID NO: 40) and htlec-lib1/2-fo (SEQ ID NO: 41) as primers using standard conditions. The ligation mixture was used to transform so-called electrocompetent *E. coli* TG-1 cells by electroporation using standard procedures. After transformation the *E. coli* TG-1 cells were plated on 2xTY-agar plates containing 0.2 mg ampicillin/mL and 2% glucose and incubated over night at 30°C.

The phage library Phtlec-1b002, containing random amino acid residues corresponding to Phtlec (SEQ ID NO: 12) positions 121-123, 125 and 126 (most of loop 1), and residues 150-153 (part of loop 4) was constructed by ligation of 20 µg *Bgl*II and *Mun*I restricted pPhtlec phagemid DNA (cf, EXAMPLE 1) with 15 µg of *Bgl*II and *Mun*I restricted DNA fragment amplified from the pair of oligonucleotides htlec-lib2-tprev (SEQ ID NO: 42) and htlec-lib2-tpfo (SEQ ID NO: 43), where N denotes a mixture of 25% of each of the nucleotides T, C, G, and A, respectively and S denotes a mixture of 50 % of C and G, encoding the appropriately randomized nucleotide sequence and the oligonucleotides htlec-lib2-rev (SEQ ID NO: 44) and htlec-lib1/2-fo (SEQ ID NO: 41) as primers using standard conditions. The ligation mixture was used to transform so-called electrocompetent *E. coli* TG-1 cells by electroporation using standard procedures. After transformation the *E. coli* TG-1 cells were plated on 2xTY-agar plates containing 0.2 mg ampicillin/mL and 2% glucose and incubated overnight at 30°C.

The titer of the libraries Phtlec-1b001 and -1b002 was determined to $1.4*10^9$ and $3.2*10^9$ clones, respectively. Six clones from each library were grown and phagemid DNA isolated using a standard miniprep procedure, and the nucleotide sequence of the loop-region determined (DNA

Technology, Aarhus, Denmark). One clone from each library failed, for technical reasons, to give reliable nucleotide sequence, and one clone from Phtlec-lib001 apparently contained a major deletion. The variation of nucleotide sequences, compared to Phtlec (SEQ ID NO: 12), of the loop-regions of the other nine clones (lb001-1, lb001-2, lb001-3, lb001-4, lb002-1, lb002-2, lb002-3, lb002-4, and lb002-5) is shown in Table 3.

Table 3: Variation of Phtlec loop derivatives isolated from the libraries Phtlec-lb001 and -lb002. (β2 and β3 consensus elements are indicated)

Clone	Loop-region sequence
Phtlec	120 130 140 150 160 β2-N D M A A E G T W V D M T G T R I A Y K N W E T E I T A Q P D G G K T E N -β3- S G A A N G K W F D - AACGACATGGCGGCCGAGGGCACCTGGGTGGACATGACCGGTACCCGCATCGCCTACAAGAACTGGGAGACTGAGATCACCGCGCAACCCGATGGCGGCAAGACCGAGAAC -- TCAGGCGCGGCCAACGGCAAGTGGTTCGAC
lb001-1	H G W R T R CACGGCTGGCGGACCCGG A N E */Q GCCAACGAGTAG V GTC
lb001-2	I */Q S E V E ATCTAGACGGAGGTCGAG D W */Q T GACTGGTAGACC G GGG
lb001-3	A G G K W R GCGGGCGGGAAGTGGCGG G G L G GGCGGCCTGGGC K AAG
lb001-4	Q R V E C G CAGAGGGTGGAGTCGGGG E A V C GAGGCGGTCTGC N AAC
lb002-1	A M S G R GGCATGAGC GGGCGG P I C R CCCATCTGCCGG
lb002-2	E A W T E GAGGCCTGG ACGGAG Q H C S CAGCACTGCTCC
lb002-3	A Q D P R GCGCAGGAC CCGCGG S L L T TCGCTCCTGACC
lb002-4	K A R K R AAGGCGCGG AAGAGG D P P P GACCCCCCCCCC
lb002-5	- - - - R P ----------CGCCCG I A R */Q ATCGCGAGGTAG

Example 5

Construction of the phage library PhtCTLD-1b003

All oligonucleotides used in this example were supplied by DNA Technology (Aarhus, Denmark).

The phage library PhtCTLD-1b003, containing random amino acid residues corresponding to PhtCTLD (SEQ ID NO: 15) positions 77 to 79 and 81 to 82 (loop 1) and 108 to 109 (loop 4) was constructed by ligation of 20 µg *Bgl*II and *Mun*I restricted pPhtCTLD phagemid DNA (cf. Example 1) with 10 µg of a *Bgl*II and *Mun*I restricted DNA fragment population encoding the appropriately randomised loop 1 and 4 regions with or without two and three random residue insertions in loop 1 and with three and four random residue insertions in loop 4. The DNA fragment population was amplified, from six so-called assembly reactions combining each of the three loop 1 DNA fragments with each of the two loop 4 DNA fragments as templates and the oligonucleotides TN-lib3-rev (SEQ ID NO: 45) and loop 3-4-5 tagfo (SEQ ID NO: 46) as primers using standard procedures. Each of the three loop 1 fragments was amplified in a reaction with either the oligonucleotides loop1b (SEQ ID NO: 47), loop1c (SEQ ID NO: 48), or loop1d (SEQ ID NO: 49) as template and the oligonucleotides TN-lib3-rev (SEQ ID NO: 45) and TN-KpnI-fo (SEQ ID NO: 50) as primers, and each of the two DNA loop 4 fragments was amplified in a reaction with either the oligonucleotide loop4b (SEQ ID NO: 51) or loop4c (SEQ ID NO: 52) as template and the oligonucleotides loop3-4rev (SEQ ID NO: 53) and loop3-4fo (SEQ ID NO: 54) as primers using standard procedures. In the oligonucleotide sequences N denotes a mixture of 25% of each of the nucleotides T, C, G, and A, respectively and S denotes a mixture of 50 % of C and G, encoding the appropriately randomized nucleotide sequence. The ligation mixture was used to transform so-called elec-

trocompetent *E. coli* TG-1 cells by electroporation using standard procedures. After transformation the E. coli TG-1 cells were plated on 2xTY-agar plates containing 0.2 mg ampicillin/mL and 2% glucose and incubated over night at 30 °C.

The size of the resulting library, PhtCTLD-1b003, was determined to $1.4*10^{10}$ clones. Twenty four clones from the library were grown and phages and phagemid DNA isolated. The nucleotide sequences of the loop-regions were determined (DNA Technology, Aarhus, Denmark) and binding to a polyclonal antibody against tetranectin, anti-TN (DAKO A/S, Denmark), analysed in an ELISA-type assay using HRP conjugated anti-gene VIII (Amersham Pharmacia Biotech) as secondary antibody using standard procedures. Eighteen clones were found to contain correct loop inserts, one clone contained the wild type loop region sequence, one a major deletion, two contained two or more sequences, and two clones contained a frameshift mutation in the region. Thirteen of the 18 clones with correct loop inserts, the wild type clone, and one of the mixed isolates reacted strongly with the polyclonal anti-TN antibody. Three of the 18 correct clones reacted weakly with the antibody, whereas, two of the correct clones, the deletion mutant, one of the mixed, and the two frameshift mutants did not show a signal above background.

Example 6

Phage selection by biopanning on anti-TN antibody.

Approximately 10^{11} phages from the PhtCTLD-1b003 library was used for selection in two rounds on the polyclonal anti-TN antibody by panning in Maxisorb immunotubes (NUNC, Denmark) using standard procedures. Fifteen clones out of $7*10^{7}$ from the plating after the second selection round

were grown and phagemid DNA isolated and the nucleotide sequence determined. All 15 clones were found to encode correct and different loop sequences.

Example 7

Model selection of CTLD-phages on plasminogen.
I: elution by trypsin digestion after panning.

In order to demonstrate that tetranectin derived CTLD bearing phages can be selected from a population of phages, mixtures of PhtCTLD phages isolated from a *E. coli* TG1 culture transformed with the phagemid pPhtCTLD (cf, EXAMPLE 1) after infection with M13K07 helper phage and phages isolated from a culture transformed with the phagemid pPhtCPB after infection with M13K07 helper phage at ratios of 1:10 and $1:10^5$, respectively were used in a selection experiment using panning in 96-well Maxisorb micro-titerplates (NUNC, Denmark) and with human plasminogen as antigen. The pPhtCPB phagemid was constructed by ligation of the double stranded oligonucleotide (SEQ ID NO: 55) with the appropriate restriction enzyme overhang sequences into KpnI and MunI restricted pPhtCTLD phagemid DNA. The pPhtCBP phages derived upon infection with the helper phages displays only the wild type M13 gene III protein because of the translation termination codons introduced into the CTLD coding region of the resulting pPhtCPB phagemid (SEQ ID NO: 56).

The selection experiments were performed in 96 well micro titer plates using standard procedures. Briefly, in each well 3 µg of human plasminogen in 100 µL PBS (PBS, 0.2 g KCl, 0.2 g KH_2PO_4, 8 g NaCl, 1.44 g Na_2HPO_4, $2H_20$, water to 1 L, and adjusted to pH 7.4 with NaOH) or 100 µL PBS (for analysis of non specific binding) was used for over night coating at 4 ^{0}C and at 37 ^{0}C for one hour. After washing once with PBS, wells were blocked with 400 µL PBS and 3%

non fat dried milk for one hour at 37°C. After blocking wells were washed once in PBS and 0.1% Tween 20 and three times with PBS before the addition of phages suspended in 100 µL PBS, 3% non fat dried milk. The phages were allowed to bind at 37 °C for one hour before washing three times with PBS, Tween 20 and three times with PBS. Bound phages were eluted from each well by trypsin digestion in 100 µL (1 mg/mL trypsin in PBS) for 30 min. at room temperature, and used for infection of exponentially growing *E. coli* TG1 cells before plating and titration on 2xTY agar plates containing 2% glucose and 0.1 mg/mL ampicillin.

Initially (round 1), 10^{12} PhtCTLD phages (A series), a mixture of 10^{10} PhtCTLD phages and 10^{11} PhtCPB phages (B series), or a mixture of 10^{6} PhtCTLD and 10^{11} PhtCPB phages (C series) were used. In the following round (round 2) 10^{11} phages of the output from each series were used. Results from the two rounds of selection are summarised in Table 4.

Table 4: Selection of mixtures of PhtCTLD and PhtCPB by panning and elution with trypsin.

		Plasminogen (*10^5 colonies)	Blank (*10^5 colonies)
Round 1	A	113.0	19.50
	B	1.8	1.10
	C	0.1	0.30
Round 2	A	49	0.10
	B	5.2	0.20
	C	0.3	0.04

Phagemid DNA from 12 colonies from the second round of plating together with 5 colonies from a plating of the ini-

tial phage mixtures was isolated and the nucleotide sequence of the CTLD region determined. From the initial 1/10 mixture (B series) of PhtCTLD/PhtCPB one out of five were identified as the CTLD sequence. From the initial $1/10^5$ mixture (C series) all five sequences were derived from the pPhtCPB phagemid. After round 2 nine of the twelve sequences analysed from the B series and all twelve sequences from the C series were derived from the pPhtCTLD phagemid.

Example 8

Model selection of CTLD-phages on plasminogen.
II: elution by 0.1 M triethylamine after panning.

In order to demonstrate that tetranectin derived CTLD-bearing phages can be selected from a population of phages, mixtures of PhtCTLD phages isolated from a *E. coli* TG1 culture transformed with the phagemid pPhtCTLD (cf, EXAMPLE 1) after infection with M13K07 helper phage and phages isolated from a culture transformed with the phagemid pPhtCPB (cf, EXAMPLE 6) after infection with M13K07 helper phage at ratios of $1:10^2$ and $1:10^6$, respectively were used in a selection experiment using panning in 96-well Maxisorb microtiterplates (NUNC, Denmark) and with human plasminogen as antigen using standard procedures.

Briefly, in each well 3 µg of human plasminogen in 100 µL PBS (PBS, 0.2 g KCl, 0.2 g KH_2PO_4, 8 g NaCl, 1.44 g Na_2HPO_4, $2H_20$, water to 1 L, and adjusted to pH 7.4 with NaOH) or 100 µL PBS (for analysis of non specific binding) was used for over night coating at 4 °C and at 37 °C for one hour. After washing once with PBS, wells were blocked with 400 µL PBS and 3% non fat dried milk for one hour at 37 °C. After blocking wells were washed once in PBS and 0.1% Tween 20 and three times with PBS before the addition of phages suspended in 100 µL PBS, 3% non fat dried milk. The phages

were allowed to bind at 37 °C for one hour before washing 15 times with PBS, Tween 20, and 15 times with PBS. Bound phages were eluted from each well by 100 µL 0.1 M triethylamine for 10 min at room temperature, and upon neutralisation with 0.5 vol. 1 M Tris-HCl pH 7.4, used for infection of exponentially growing *E. coli* TG1 cells before plating and titration on 2xTY agar plates containing 2% glucose and 0.1 mg/mL ampicillin.

Initially (round 1) 10^{12} PhtCTLD phages (A series), a mixture of 10^{9} PhtCTLD phages and 10^{11} PhtCPB phages (B series), or a mixture of 10^{5} PhtCTLD and 10^{11} PhtCPB phages (C series) were used. In the following round (round 2) 10^{11} phages of the output from each series were used. Results from the two rounds of selection are summarised in Table 5.

Table 5: Selection of mixtures of PhtCTLD and PhtCPB by panning elution with triethylamine.

		Plasminogen (*10^4 colonies)	Blank (*10^4 colonies)
Round 1	A	18	0.02
	B	0.5	0.00
	C	0.25	0.02
Round 2	A	n.d.	n.d.
	B	5.0	0.00
	C	1.8	0.02
Round 3	A	n.d.	n.d.
	B	11	0.00
	C	6.5	0.02

n.d. = not determined

Phage mixtures from the A and the B series from the second round of selection were grown using a standard procedure, and analysed for binding to plasminogen in an ELISA-type assay. Briefly, in each well 3 µg of plasminogen in 100 µL PBS (PBS, 0.2 g KCl, 0.2 g KH2PO4, 8 g NaCl, 1.44 g Na_2HPO_4, $2H_20$, water to 1 L, and adjusted to pH 7.4 with NaOH) or 100 µL PBS (for analysis of non specific binding) was used for over night coating at 4 °C and at 37 °C for one hour. After washing once with PBS, wells were blocked with 400 µL PBS and 3% non fat dried milk for one hour at 370C. After blocking wells were washed once in PBS and 0.1% Tween 20 and three times with PBS before the addition of phages suspended in 100 µL PBS, 3% non fat dried milk. The phage mixtures were allowed to bind at 37 °C for one hour before washing three times with PBS, Tween 20, and three times with PBS. After washing, 50 µL of a 1:5000 dilution of a HRP-conjugated anti-gene VIII antibody (Amersham Pharmacia Biotech) in PBS, 3% non fat dried milk was added to each well and incubated at 37 °C for one hour. After binding of the "secondary" antibody wells were washed three times with PBS, Tween 20, and three times with PBS before the addition of 50 µL of TMB substrate (DAKO-TMB One-Step Substrate System, code: S1600, DAKO, Denmark). Reaction was allowed to proceed for 20 min. before quenching with 0.5 vol. 0.5 M H_2SO_4, and analysis. The result of the ELISA analysis confirmed specific binding to plasminogen of phages in both series (fig. 28).

Example 9

<u>Selection of phages from the library Phtlec-1b002 binding to hen egg white lysozyme</u>.

$1.2*10^{12}$ phages, approximately 250 times the size of the original library, derived from the Phtlec-1b002 library

(cf, EXAMPLE 4) were used in an experimental procedure for the selection of phages binding to hen egg white lysozyme involving sequential rounds of panning using standard procedures.

Briefly, 30 µg of hen egg white lysozyme in 1 mL PBS (PBS, 0.2 g KCl, 0.2 g KH_2PO_4, 8 g NaCl, 1.44 g Na_2HPO_4, $2H_20$, water to 1 L, and adjusted to pH 7.4 with NaOH) or 1 mL PBS (for analysis of non specific binding) was used for over night coating of Maxisorb immunotubes (NUNC, Denmark) at 4 ^{0}C and at 37 ^{0}C for one hour. After washing once with PBS, tubes were filled and blocked with PBS and 3% non fat dried milk for one hour at 37^{0}C. After blocking tubes were washed once in PBS, 0.1% Tween 20 and three times with PBS before the addition of phages suspended in 1 mL PBS, 3% non fat dried milk. The phages were allowed to bind at 37 ^{0}C for one hour before washing six times with PBS, Tween 20 and six times with PBS. Bound phages were eluted from each well by 1 mL 0.1 M triethylamine for 10 min at room temperature, and upon neutralisation with 1 M Tris-HCl pH 7.4, used for infection of exponentially growing *E. coli* TG1 cells before plating and titration on 2xTY agar plates containing 2% glucose and 0.1 mg/mL ampicillin. In the subsequent rounds of selection approximately 10^{12} phages derived from a culture grown from the colonies plated after infection with the phages eluted from the lysozyme coated tube were used in the panning procedure. However, the stringency in binding was increased by increasing the number of washing step after phage panning from six to ten.

The results from the selection procedure is shown in Table 7.

Table 7: Selection by panning of lysozyme binding phages from Phtlec-1b002 library.

	Lysozyme	Blank	Ratio
Round 1	$2.4*10^4$	n.a.	n.a.
Round 2	$3.5*10^3$	$4.0*10^2$	9
Round 3	$3.2*10^5$	$2.5*10^2$	$1.3*10^3$

n.a. = not applicable

Phages were grown from twelve clones isolated from the third round of selection in order to analyse the specificity of binding using a standard procedure, and analysed for binding to hen egg white lysozyme and human β_2-microglobulin in an ELISA-type assay. Briefly, in each well 3 µg of hen egg white lysozyme in 100 µL PBS (PBS, 0.2 g KCl, 0.2 g KH_2PO_4, 8 g NaCl, 1.44 g Na_2HPO_4, $2H_20$, water to 1 L, and adjusted to pH 7.4 with NaOH), or 3 µg of human β_2-microglobulin, or 100 µL PBS (for analysis of non specific binding) was used for over night coating at 4 °C and at 37 °C for one hour. After washing once with PBS, wells were blocked with 400 µL PBS and 3% non fat dried milk for one hour at 37°C. After blocking wells were washed once in PBS and 0.1% Tween 20 and three times with PBS before the addition of phages suspended in 100 µL PBS, 3% non fat dried milk. The phages were allowed to bind at 37 °C for one hour before washing three times with PBS, Tween 20 and three times with PBS. After washing, 50 µL of a 1 to 5000 dilution of a HRP-conjugated anti-gene VIII antibody (Amersham Pharmacia Biotech) in PBS, 3% non fat dried milk was added to each well and incubated at 37 °C for one hour. After binding of the "secondary" antibody wells were washed three times with PBS, Tween 20 and three times with PBS before the addition of 50 µL of TMB substrate (DAKO-TMB One-Step Substrate System, code: S1600, DAKO, Denmark). Reac-

tion was allowed to proceed for 20 min before quenching with 0.5 M H_2SO_4.

Results showing relatively weak but specific binding to lysozyme are summarised in Fig. 29.

EXAMPLE 10

Construction of the rat mannose-binding protein CTLD (r-MBP) derived phagemid (pPrMBP) and human lung surfactant protein D CTLD (h-SP-D) derived phagemid (pPhSP-D)

The phagemid, pPrMBP, is constructed by ligation of the *Sfi* I and *Not* I restricted DNA fragment amplified from cDNA, isolated from rat liver (Drickamer, K., et al., *J. Biol. Chem.* 1987, 262(6):2582-2589) (with the oligonucleotide primers SfiMBP 5'-CGGCTGAGCGGCCCAGCCGGCCATGGC-CGAGCCAAACAAGTTGCATGCCTTCTCC-3' [SEQ ID NO:62] and NotMBP 5'-GCACTCCTGCGGCCGCGGCTGGGAACTCGCAGAC-3' [SEQ ID NO:63]) into a Sfi I and Not I precut vector, pCANTAB 5E supplied by Amersham Pharmacia Biotech (code no. 27-9401-01) using standard procedures. Outlines of the resulting pPrMBP is shown in Fig. 31 and the nucleotide sequence of PrMBP is given as (SEQ ID NO:58). The amino acid sequence encoded by the PrMBP insert is shown in Fig. 30 (SEQ ID NO:59).

The phagemid,pPhSP-D, is constructed by ligation of the *Sfi* I and *Not* I restricted DNA fragment amplified from cDNA, isolated from human lung (Lu,J., et al., *Biochem J.* 1992 jun 15; 284:795-802) (with the oligonucleotide primers SfiSP-D 5'-CGGCTGAGCGGCCCAGCCGGCCATGGCCGAGCCAAAGAAAGTTGA-GCTCTTCCC-3' [SEQ ID NO:64] and NotSP-D 5'-GCACTCCTGCGGC-CGCGAACTCGCAGACCACAAGAC-3' [SEQ ID NO:65]) into a *Sfi* I and *Not* I precut vector, pCANTAB 5E supplied by Amersham Pharmacia Biotech (code no. 27-9401-01) using standard procedures. Outlines of the resulting pPhSP-D is shown in Fig.

33 and the nucleotide sequence of PhSP-D, is given as (SEQ ID NO:60). The amino acid sequences encoded by the PhSP-D insert is shown in Fig 32 (SEQ ID NO:61).

Example 11

Construction of the phage library PrMBP-1b001

The phage library PrMBP-1b001, containing random amino acid residues corresponding to PrMBP CTLD (SEQ ID NO:59) positions 71 to 73 or 70 to 76 (loop 1) and 97 to 101 or 100 to 101 (loop 4) is constructed by ligation of 20 µg *Sfi*I and *Not*I restricted pPrMBP phagemid DNA (cf. Example 10) with 10 µg of a *Sfi*I and *Not*I restricted DNA fragment population encoding the appropriately randomised loop 1 and 4 regions. The DNA fragment population is amplified, from nine assembly reactions combining each of the three loop 1 DNA fragments with each of the three loop 4 DNA fragments as templates and the oligonucleotides Sfi-tag 5'-CGGCTGAGCGGCCCAGC-3' (SEQ ID NO:74) and Not-tag 5'-GCACTCCTGCGGCCGCG-3' (SEQ ID NO:75) as primers using standard procedures. Each of the three loop 1 fragments is amplified in a primary PCR reaction with pPrMBP phagmid DNA (cf. Example 10) as template and the oligonucleotides MBPloop1a fo (SEQ ID NO:66), MBPloop1b fo (SEQ ID NO:67)or MBPloop1c fo (SEQ ID NO:68) and SfiMBP (SEQ ID NO:62) as primers, and further amplified in a secondary PCR reaction using Sfi-tag (SEQ ID NO:74) and MBPloop1-tag fo (SEQ ID NO:69). Each of the three DNA loop 4 fragments is amplified in a primary PCR reaction with pPrMBP phagemid DNA (cf. Example 10) as template and the oligonucleotides MBPloop4a rev (SEQ ID NO:71), MBPloop4b rev (SEQ ID NO:72) or MBPloop4c rev (SEQ ID NO:73) and NotMBP (SEQ ID NO:63) as primers using standard procedures and further amplified in a secondary PCR reaction using MBPloop4-tag rev (SEQ ID NO:70) and Not-tag

(SEQ ID NO:63). In the oligonucleotide sequences N denotes a mixture of 25% of each of the nucleotides T, C, G, and A, respectively, and S denotes a mixture of 50 % of C and G, encoding the appropriately randomized nucleotide sequence. The ligation mixture is used to transform so-called electrocompetent *E. coli* TG-1 cells by electroporation using standard procedures. After transformation the *E. coli* TG-1 cells are plated on 2xTY-agar plates containing 0.2 mg ampicillin/mL and 2% glucose and incubated over night at 30 °C.

Example 12

Construction of the phage library PhSP-D-1b001

The phage library PhSP-D-1b001, containing random amino acid residues corresponding to PhSP-D CTLD insert (SEQ ID NO:61) positions 74 to 76 or 73 to 79 (loop 1) and 100 to 104 or 103 to 104 (loop 4) is constructed by ligation of 20 µg *Sfi*I and *Not*I restricted pPhSP-D phagemid DNA (cf. Example 10) with 10 µg of a *Sfi*I and *Not*I restricted DNA fragment population encoding the appropriately randomised loop 1 and 4 regions. The DNA fragment population is amplified, from nine assembly reactions combining each of the three loop 1 DNA fragments with each of the three loop 4 DNA fragments as templates and the oligonucleotides Sfi-tag 5'-CGGCTGAGCGGCCCAGC-3' (SEQ ID NO:74) and Not-tag 5'-GCACTCCTGCGGCCGCG-3' (SEQ ID NO:75) as primers using standard procedures. Each of the three loop 1 fragments is amplified in a primary PCR reaction with pPhSP-D phagemid DNA (cf. Example 10) as template and the oligonucleotides Sp-dloop1a fo (SEQ ID NO:76), Sp-dloop1b fo (SEQ ID NO:77)or Sp-dloop1c fo (SEQ ID NO:78) and SfiSP-D (SEQ ID NO:64) as primers, and further amplified in a PCR reaction using Sfi-tag (SEQ ID NO:74) and Sp-dloop1-tag fo (SEQ ID NO:79) as

primers. Each of the three DNA loop 4 fragments is amplified in a primary PCR reaction with pPhSP-D phagemid DNA (cf. Example 10) as template and the oligonucleotides Sp-dloop4a rev (SEQ ID NO:81), Sp-dloop4b rev (SEQ ID NO:82) or Sp-dloop4c rev (SEQ ID NO:83) and NotSP-D (SEQ ID NO:65) as primers using standard procedures and further amplified in a PCR reaction using Sp-dloop4-tag rev (SEQ ID NO:80) and Not-tag (SEQ ID NO:75) as primers. In the oligonucleotide sequences N denotes a mixture of 25% of each of the nucleotides T, C, G, and A, respectively, and S denotes a mixture of 50 % of C and G, encoding the appropriately randomized nucleotide sequence. The ligation mixture is used to transform so-called electrocompetent *E. coli* TG-1 cells by electroporation using standard procedures. After transformation the *E. coli* TG-1 cells are plated on 2xTY-agar plates containing 0.2 mg ampicillin/mL and 2% glucose and incubated over night at 30 °C.

Example 13

Construction of the phage library PhtCTLD-1b004

All oligonucleotides used in this example were supplied by DNA Technology (Aarhus, Denmark).

The phage library PhtCTLD-1b004, containing random amino acid residues corresponding to PhtCTLD (SEQ ID NO:15) positions 97 to 102 or 98 to 101(loop 3) and positions 116 to 122 or 118 to 120 (loop 5) was constructed by ligation of 20 µg *Kpn*I and *Mun*I restricted pPhtCTLD phagemid DNA (cf. Example 1) with 10 µg of a *Kpn*I and *Mun*I restricted DNA fragment population encoding the randomised loop 3 and 5 regions. The DNA fragment population was amplified from nine primary PCR reactions combining each of the three loop 3 DNA fragments with each of the three loop 5 DNA fragments. The fragments was amplified with either of the oli-

gonucleotides loop3a (SEQ ID NO:84), loop3b (SEQ ID NO: 85), or loop3c (SEQ ID NO:86) as template and loop5a(SEQ ID NO:87), loop5b(SEQ ID NO:88)or loop5c(SEQ ID NO:89) and loop3-4rev(SEQ ID NO:91) as primers. The DNA fragments were further amplified in PCR reactions, using the primary PCR product as template and the oligonucleotide loop3-4rev (SEQ ID NO:91) and loop3-4-5tag fo (SEQ ID NO:90) as primers. All PCR reactions were performed using standard procedures.

In the oligonucleotide sequences N denotes a mixture of 25% of each of the nucleotides T, C, G, and A, respectively and S denotes a mixture of 50 % of C and G, encoding the appropriately randomised nucleotide sequence. The ligation mixture was used to transform so-called electrocompetent E. coli TG-1 cells by electroporation using standard procedures. After transformation the E. coli TG-1 cells were plated on 2xTY-agar plates containing 0.2 mg ampicillin/mL and 2% glucose and incubated over night at 30 °C.

The size of the resulting library, PhtCTLD-1b004, was determined to 7*109 clones. Sixteen clones from the library were picked and phagemid DNA isolated. The nucleotide sequence of the loop-regions were determined (DNA Technology, Aarhus, Denmark). Thirteen clones were found to contain correct loop inserts and three clones contained a frameshift mutation in the region.

Example 14

Selection of Phtlec-phages and PhtCTLD-phages binding to the blood group A sugar moiety immobilised on human serum albumin

Phages grown from glycerol stocks of the libraries Phtlec-1b001 and Phtlec-1b002 (cf. Example 4) and phages grown from a glycerol stock of the library PhtCTLD-1b003 (cf. Ex-

ample 5), using a standard procedure, were used in an experiment designed for the selection of Phtlec- and PhtCTLD derived phages with specific affinity to the blood group A sugar moiety immobilized on human serum albumin, A-HA, by panning in 96-well Maxisorb micro-titerplates (NUNC, Denmark) using standard procedures.

Initially, the phage supernatants were precipitated with 0.3 vol. of a solution of 20% polyethylene glycol 6000 (PEG) and 2.5 M NaCl, and the pellets re-suspended in TE-buffer (10 mM Tris-HCl pH 8, 1 mM EDTA). After titration on E. coli TG-1 cells, phages derived from Phtlec-1b001 and -1b002 were mixed (#1) in a 1:1 ratio and adjusted to 5*1012 pfu/mL in 2*TY medium, and phages grown from the PhtCTLD-1b003 library (#4) were adjusted to 2.5*1012 pfu/mL in 2*TY medium.

One microgram of the "antigen", human blood group A trisaccharide immobilised on human serum albumin, A-HA, (Glycorex AB, Lund, Sweden) in 100 µL PBS (PBS, 0.2 g KCl, 0.2 g KH_2PO_4, 8 g NaCl, 1.44 g Na_2HPO_4, $2H_20$, water to 1 L, and adjusted to pH 7.4 with NaOH), in each of three wells, was coated over night at 4 °C and at room temperature for one hour, before the first round of panning. After washing once with PBS, wells were blocked with 300 µL PBS and 3% non fat dried milk for one hour at room temperature. After blocking wells were washed once in PBS and 0.1% Tween 20 and three times with PBS before the addition of a mixture of 50 µL of the phage suspension and 50 µL PBS, 6% non fat dried milk. The phages were allowed to bind at room temperature for two hours before washing eight times with PBS, Tween 20, and eight times with PBS. Bound phages were eluted from each well by trypsin digestion in 100 µL (1 mg/mL trypsin in PBS) for 30 min. at room temperature, and used for infection of exponentially growing *E. coli* TG1 cells before

plating and titration on 2xTY agar plates containing 2% glucose and 0.1 mg/mL ampicillin.

In the second round of selection, 150 µL of crude phage supernatant, grown from the first round output colonies, was mixed with 150 µL PBS, 6% non fat dried milk, and used for panning distributing 100 µL of the mixture in each of three A-HA coated wells, as previously described. Stringency in binding was increased by increasing the number of washing steps from 16 to 32. 300 µL of phage mixture was also used for panning in three wells, which had received no antigen as control.

In the third round of selection, 150 µL of crude phage supernatant, grown from the second round output colonies, was mixed with 150 µL PBS, 6% non fat dried milk, and used for panning distributing 100 µL of the mixture in each of three A-HA coated wells, as previously described. The number of washing steps was again 32. 300 µL of phage mixture was also used for panning in three wells, which had received no antigen as control.

The results from the selection procedure are summarised in Table 8

Table 8. Selection of Phtlec phages (#1) and PhtCTLD phages (#4) binding to A-HA by panning and elution with trypsin digestion.

		A-HA	Blank	Ratio
Round 1	#1	$0.8*10^3$	n.a.	n.a.
	#4	$1.1*10^3$	n.a.	n.a.
Round 2	#1	$1.0*10^3$	$0.5*10^2$	20
	#4	$1.3*10^3$	$0.5*10^2$	26
Round 3	#1	$8.0*10^4$	$0.5*10^2$	1600
	#4	$9.0*10^5$	$0.5*10^2$	18000

n.a. not applicable.

48 clones from each of the #1 and #4 series were picked and grown in a 96 well microtiter tray and phages produced by infection with M13K07 helper phage using a standard procedure. Phages from the 96 phage supernatants were analysed for binding to the A-HA antigen and for non-specific binding to hen egg white lysozyme using an ELISA-type assay. Briefly, in each well 1 µg of A-HA in 100 µL PBS (PBS, 0.2 g KCl, 0.2 g KH_2PO_4, 8 g NaCl, 1.44 g Na_2HPO_4, $2H_20$, water to 1 L, and adjusted to pH 7.4 with NaOH) or 1 µg of hen egg white lysozyme in 100 µL PBS (for analysis of non specific binding) was used for over night coating at 4 °C and at room temperature for one hour. After washing once with PBS, wells were blocked with 300 µL PBS and 3% non fat dried milk for one hour at room temperature. After blocking wells were washed once in PBS and 0.1% Tween 20 and three times with PBS before the addition of 50 µL phage supernatant in 50 µL PBS, 6% non fat dried milk. The phage mixtures were allowed to bind at room temperature for two hours before washing three times with PBS, Tween 20, and three times with PBS. After washing, 50 µL of a 1:5000 di-

lution of a HRP-conjugated anti-gene VIII antibody (Amersham Pharmacia Biotech) in PBS, 3% non fat dried milk, was added to each well and incubated at room temperature for one hour. After binding of the "secondary" antibody wells were washed three times with PBS, Tween 20, and three times with PBS before the addition of 50 µL of TMB substrate (DAKO-TMB One-Step Substrate System, DAKO, Denmark). Reaction was allowed to proceed for 20 min. before quenching with 0.5 M H_2SO_4, and analysis. The result of the ELISA analysis showed "hits" in terms of specific binding to A-HA of phages in both series (fig. 34 and 35), as judged by a signal ratio between signal on A-HA to signal on lysozyme at or above 1.5, and with a signal above background.

From the #1 series 13 hits were identified and 28 hits were identified from the #4 series.

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CLAIMS

1. A protein having the scaffold structure of C-type lectin-like domains (CTLD), said protein comprising a variant of a model CTLD wherein the α-helices and β-strands and connecting segments are conserved to such a degree that the scaffold structure of the CTLD is substantially maintained, while the loop region is altered by amino acid substitution, deletion, insertion or any combination thereof, with the proviso that said protein is not any of the known CTLD loop derivatives of C-type lectin-like proteins or C-type lectins listed in Table 2 in the description.

2. A protein according to claim 1 wherein the model CTLD is defined by having a 3D structure that conforms to the secondary-structure arrangement illustrated in Fig 1 characterized by the following main secondary structure elements:

five β-strands and two α-helices sequentially appearing in the order β1, α1, α2, β2, β3, β4, and β5, the β-strands being arranged in two anti-parallel β-sheets, one composed of β1 and β5, the other composed of β2, β3 and β4,

at least two disulfide bridges, one connecting α1 and β5 and one connecting β3 and the polypeptide segment connecting β4 and β5, and

a loop region consisting of two polypeptide segments, loop segment A (LSA) connecting β2 and β3 and comprising typically 15-70 or, less typically, 5-14 amino acid residues, and loop segment B (LSB) connecting β3 and β4 and comprising typically 5-12 or less typically, 2-4 amino acid residues.

3. A protein according to claim 1 wherein the model CTLD is defined by showing sequence similarity to a previously rec-

ognised member of the CTLD family as expressed by an amino acid sequence identity of at least 22 %, preferably at least 25 % and more preferably at least 30 %, and by containing the cysteine residues necessary for establishing the conserved two-disulfide bridge topology (i.e. Cys_I, Cys_{II}, Cys_{III} and Cys_{IV}), whereas the loop region and its flanking β-strand structural elements are identified by inspection of the sequence alignment with the collection of CTLDs shown in Fig. 1 providing identification of the sequence locations of the β2- and β3-strands with the further corroboration provided by comparison of these sequences with the four-residue consensus sequences, β2cseq and β3cseq, the β4 strand segment being located typically at positions -6 to -2 and less typically at positions -5 to -2 relative to the conserved Cys_{III} residue and with the characteristic residues at positions -5 and -3 as elucidated from Table 1 and deducted in the description.

4. A protein according to any one of the preceding claims wherein up to 10, preferably up to 4, and more preferably 1 or 2, amino acid residues are substituted, deleted or inserted in the α-helices and/or β-strands and/or connecting segments of the model CTLD.

5. A protein according to any one of the preceding claims wherein changes of up to 4 residues are made in the β-strands of the model CTLD as a consequence of the introduction of recognition sites for one or more restriction endonucleases in the nucleotide sequence encoding the CTLD to facilitate the excision of part or all of the loop region and the insertion of an altered amino acid sequence instead while the scaffold structure of the CTLD is substantially maintained.

6. A protein according to any one of the preceding claims wherein the model CTLD is that of a tetranectin.

7. A protein according to claim 6 wherein the model CTLD is that of human tetranectin comprising amino acid residues 59 to 180 in the monomer native peptide sequence thereof.

8. A protein according to claim 6 wherein the model CTLD is that of murine tetranectin comprising amino acid residues 59 to 180 in the monomer native peptide sequence thereof.

9. A protein according to any one of the preceding claims which further comprises N-terminal and/or C-terminal extensions of the CTLD variant.

10. A protein according to claim 9 wherein said N-terminal and/or C-terminal extensions contain effector, enzyme, further binding and/or multimerising functions.

11. A protein according to claim 9 or 10 wherein said N-terminal and/or C-terminal extensions are the non-CTLD-portions of a native C-type lectin-like protein or C-type lectin or a "soluble" variant thereof lacking a functional transmembrane domain.

12. A protein according to any one of the preceding claims which is a multimer of a moiety comprising the CTLD variant.

13. A protein according to claim 12 which is derived from the native tetranectin trimer.

14. A protein according to claim 7 which is derived from the polypeptide htlec having the amino acid sequence from position 5 Glu to position 185 Val in SEQ IN NO:13 by altering the amino acid sequence of the loop region.

15. A protein according to claim 7 which is derived from the polypeptide htCTLD having the amino acid sequence from position 5 Ala to position 141 Val in SEQ IN NO:15 by altering the amino acid sequence of the loop

16. A protein according to claim 7 which is derived from the polypeptide hTN having the amino acid sequence from position 5 Glu to position 185 Val in SEQ IN NO:09 by altering the amino acid sequence of the loop region.

17. A protein according to claim 7 which is derived from the polypeptide hTN3 having the amino acid sequence from position 5 Ala to position 141 Val in SEQ IN NO:11 by altering the amino acid sequence of the loop region.

18. A protein according to claim 8 which is derived from the polypeptide mtlec having the amino acid sequence from position 5 Glu to position 185 Val in SEQ IN NO:36 by altering the amino acid sequence of the loop region.

19. A protein according to claim 8 which is derived from the polypeptide mtCTLD having the amino acid sequence from position 5 Ala to position 141 Val in SEQ IN NO:38 by altering the amino acid sequence of the loop region.

20. A combinatorial library of proteins having the scaffold structure of C-type lectin-like domains (CTLD), said proteins comprising variants of a model CTLD wherein the α-helices and β-strands are conserved to such a degree that the scaffold structure of the CTLD is substantially maintained, while the loop region or parts of the loop region of the CTLD is randomised with respect to amino acid sequence and/or number of amino acid residues.

21. A combinatorial library according to claim 20 wherein the model CTLD is defined by having a 3D structure that conforms to the secondary-structure arrangement illustrated in Fig. 1 characterised by the following main secondary structure elements:

five β-strands and two α-helices sequentially appearing in the order β1, α1, α2, β2, β3, β4, and β5, the β-

strands being arranged in two anti-parallel β-sheets, one composed of β1 and β5, the other composed of β2, β3 and β4,

at least two disulfide bridges, one connecting α1 and β5 and one connecting β3 and the polypeptide segment connecting β4 and β5, and

a loop region consisting of two polypeptide segments, loop segment A (LSA) connecting β2 and β3 and comprising typically 15-70 or, less typically, 5-14 amino acid residues, and loop segment B (LSB) connecting β3 and β4 and comprising typically 5-12 or less typically, 2-4 amino acid residues.

22. A combinatorial library according to claim 20 wherein the model CTLD is defined by showing sequence similarity to a previously recognised member of the CTLD family as expressed by an amino acid sequence identity of at least 22 %, preferably at least 25 % and more preferably at least 30 %, and by containing the cysteine residues necessary for establishing the conserved two-disulfide bridge topology (i.e. Cys_I, Cys_{II}, Cys_{III} and Cys_{IV}), whereas the loop region and its flanking β-strand structural elements are identified by inspection of the sequence alignment with the collection of CTLDs shown in Fig. 1 providing identification of the sequence locations of the β2- and β3-strands with the further corroboration provided by comparison of these sequences with the four-residue consensus sequences, β2cseq and β3cseq, the β4 strand segment being located typically at positions -6 to -2 and less typically at positions -5 to -2 relative to the conserved Cys_{III} residue and with the characteristic residues at positions -5 and -3 as elucidated from Table 1 and deducted in the description.

23. A combinatorial library according to any one of claims 20-22 of proteins comprising CTLD variants wherein up to

10, preferably up to 4, and more preferably 1 or 2, amino acid residues are substituted, deleted or inserted in the α-helices and β-strands and connecting segments of the model CTLD.

24. A combinatorial library according to any one of claims 20-23 of proteins comprising CTLD variants wherein changes of up to 4 residues are made in the model CTLD as a consequence of the introduction of recognition sites for one or more restriction endonucleases in the nucleotide sequence encoding the CTLD to facilitate the excision of part or all of a DNA segment encoding the loop region and the insertion of members of an ensemble of DNA fragments that collectively encode a randomised amino acid sequence instead while the scaffold structure of the CTLD is substantially maintained.

25. A combinatorial library according to any one of claims 20-24 of proteins wherein the model CTLD is that of a tetranectin.

26. A combinatorial library according to claim 25 of proteins wherein the model CTLD is that of human tetranectin comprising amino acid residues 59 to 180 in the monomer native peptide sequence thereof.

27. A combinatorial library according to claim 25 of proteins wherein the model CTLD is that of murine tetranectin comprising amino acid residues 59 to 180 in the monomer native peptide sequence thereof.

28. A combinatorial library according to any one of claims 20-27 of proteins which further comprise N-terminal and/or C-terminal extensions of the CTLD-variant.

29. A combinatorial library according to claim 28 of proteins wherein said N-terminal and/or C-terminal extensions

contain effector, enzyme, further binding and/or multimerising functions.

30. A combinatorial library according to claim 28 or 29 of proteins wherein said N-terminal and/or C-terminal extensions are the non-CTLD-portions of a native C-type lectin-like protein or C-type lectin or a "soluble" variant thereof lacking a functional transmembrane domain.

31. A combinatorial library according to any one of claims 20-30 of proteins which are multimers of a moiety comprising the CTLD variant.

32. A combinatorial library according to claim 31 of proteins which are derived from the native tetranectin trimer.

33. A combinatorial library according to claim 27 of proteins which are derived from the peptide htlec having the amino acid sequence from position 5 Glu to position 185 Val in SEQ IN NO:13 by randomising the loop region with respect to amino acid sequence and/or number of amino acid residues.

34. A combinatorial library according to claim 27 of proteins which are derived from the peptide htCTLD having the amino acid sequence from position 5 Ala to position 141 Val in SEQ IN NO:15 by randomising the loop region with respect to amino acid sequence and/or number of amino acid residues.

35. A combinatorial library according to claim 27 of proteins which are derived from the peptide hTN having the amino acid sequence from position 5 Glu to position 185 Val in SEQ IN NO:09 by randomising the loop region with respect to amino acid sequence and/or number of amino acid residues.

36. A combinatorial library according to claim 27 of proteins which are derived from the peptide hTN3 having the amino acid sequence from position 5 Ala to position 141 Val in SEQ IN NO:11 by randomising the loop region with respect to amino acid sequence and/or number of amino acid residues.

37. A combinatorial library according to claim 28 of proteins which are derived from the peptide mtlec having the amino acid sequence from position 5 Glu to position 185 Val in SEQ IN NO:36 by randomising the loop region with respect to amino acid sequence and/or number of amino acid residues.

38. A combinatorial library according to claim 28 of proteins which are derived from the peptide mtCTLD having the amino acid sequence from position 5 Ala to position 141 Val in SEQ IN NO:38 by randomising the loop region with respect to amino acid sequence and/or number of amino acid residues.

39. A derivative of a native tetranectin wherein up to 10, preferably up to 4, and more preferably 1 or 2, amino acid residues are substituted, deleted or inserted in the α-helices and/or β-strands and/or connecting segments of its CTLD with the proviso that said derivative is not any of the known CTLD derivatives of human tetranectin (hTN) listed in Table 2 in the description.

40. A derivative of human tetranectin, termed htlec, having the amino acid sequence from position 5 Glu to position 185 Val in SEQ IN NO:13.

41. A derivative of human tetranectin, termed htCTLD, having the amino acid sequence from position 5 Ala to position 141 Val in SEQ IN NO:15.

42. A derivative of human tetranectin, termed PhTN, having the amino acid sequence given in SEQ IN NO:09.

43. A derivative of human tetranectin, termed PhTN3, having the amino acid sequence given in SEQ IN NO:11.

44. A derivative of human tetranectin, termed Phtlec, having the amino acid sequence given in SEQ IN NO:13.

45. A derivative of human tetranectin, termed PhtCTLD, having the amino acid sequence given in SEQ IN NO:15.

46. A derivative of human tetranectin, termed FX-htlec, having the amino acid sequence given in SEQ IN NO:02.

47. A derivative of human tetranectin, termed FX-htCTLD, having the amino acid sequence given in SEQ IN NO:04.

48. A derivative of murine tetranectin, termed mtlec, having the amino acid sequence from position 5 Glu to position 185 Val in SEQ IN NO:36.

49. A derivative of murine tetranectin, termed mtCTLD, having the amino acid sequence from position 5 Ala to position 141 Val in SEQ IN NO:38.

50. A derivative of murine tetranectin, termed Pmtlec, having the amino acid sequence given in SEQ IN NO:36.

51. A derivative of murine tetranectin, termed PmtCTLD, having the amino acid sequence given in SEQ IN NO:38.

52. A derivative of murine tetranectin, termed FX-mtlec, having the amino acid sequence given in SEQ IN NO:29.

53. A derivative of murine tetranectin, termed FX-mtCTLD, having the amino acid sequence given in SEQ IN NO:31.

54. Nucleic acid comprising a nucleotide sequence encoding a htlec insert as shown from nucleotide 20 to nucleotide 562 in SEQ ID NO:12.

55. Nucleic acid comprising a nucleotide sequence encoding a htCTLD insert as shown from nucleotide 20 to nucleotide 430 in SEQ ID NO:14.

56. Nucleic acid comprising a nucleotide sequence encoding a mtlec insert as shown from nucleotide 20 to nucleotide 562 in SEQ ID NO:35.

57. Nucleic acid comprising a nucleotide sequence encoding a mtCTLD insert as shown from nucleotide 20 to nucleotide 430 in SEQ ID NO:37.

58. Nucleic acid comprising any nucleotide sequence encoding a protein according to any one of claims 1-19.

59. A library of nucleic acids encoding proteins of a combinatorial library according to any one of claims 20-38, in which the members of the ensemble of nucleic acids, that collectively constitute said library of nucleic acids, are able to be expressed in a display system, which provides for a logical, physical or chemical link between entities displaying phenotypes representing properties of the displayed expression products and their corresponding genotypes.

60. A library of nucleic acids according to claim 49, wherein the display system is selected from

(I) a phage display system such as

(1) a filamentous phage fd in which the library of nucleic acids is inserted into

(a) a phagemid vector,

(b) the viral genome of a phage

(c) purified viral nucleic acid in purified single- or double-stranded form, or

(2) a phage lambda in which the library is inserted into

(a) purified phage lambda DNA, or

(b) the nucleic acid in lambda phage particles; or

(II) a viral display system in which the library of nucleic acids is inserted into the viral nucleic acid of a eukaryotic virus such as baculovirus; or

(III) a cell-based display system in which the library of nucleic acids is inserted into, or adjoined to, a nucleic acid carrier able to integrate either into the host genome or into an extrachromosomal element able to maintain and express itself within the cell and suitable for cell-surface display on the surface of

(a) bacterial cells,

(b) yeast cells, or

(c) mammalian cells; or

(IV) a nucleic acid entity suitable for ribosome linked display into which the library of nucleic acid is inserted; or

(V) a plasmid suitable for plasmid linked display into which the library of nucleic acid is inserted.

61. A library of nucleic acids according to claim 60 wherein said phagemid vector is the vector "pCANTAB 5 E" supplied by Amersham Pharmacia Biotech (code no. 27-9401-01) for use with their "Recombinant Phage Antibody System".

62. A method of preparing a protein according to any one of claims 1-19, wherein the protein comprises at least one or

more, identical or not identical, CTLD domains with novel loop-region sequences which has (have) been isolated from one or more CTLD libraries by screening or selection.

63. A method of preparing a protein according to claim 62, wherein at least one CTLD domain has been further modified by mutagenesis.

64. A method of preparing a protein according to claim 62 or 63, wherein the protein containing at least one CTLD domain is assembled from two or more components by chemical or enzymatic coupling or crosslinking.

65. A method of preparing a combinatorial library according to any one of claims 20-38 comprising the following steps:

1) inserting nucleic acid encoding a protein comprising a model CTLD into a suitable vector,
2) if necessary, introducing restriction endonuclease recognition sites by site directed mutagenesis, said recognition sites being properly located in the sequence at or close to the ends of the sequence encoding the loop region of the CTLD or part thereof,
3) excising the DNA fragment encoding the loop region or part thereof by use of the proper restriction endonucleases,
4) ligating mixtures of DNA fragments into the restricted vector, and
5) inducing the vector to express randomised proteins having the scaffold structure of CTLDs in a suitable medium.

66. A method of constructing a tetranectin derivative adapted for the preparation of a combinatorial library according to any one of claims 20-38, wherein the nucleic acid encoding the tetranectin derivative has been modified to generate endonuclease restriction sites within nucleic acid segments encoding β2, β3 or β4, or up to 30 nucleo-

tides upstream or downstream in the sequence from any nucleotide which belongs to a nucleic acid segment encoding β2, β3or β4.

67. The use of a nucleotide sequence encoding a tetranectin, or a derivative thereof wherein the scaffold structure of its CTLD is substantially maintained, for preparing a library of nucleotide sequences encoding related proteins by randomising part or all of the nucleic acid sequence encoding the loop region of its CTLD.

68. The use according to claim 67 wherein the nucleotide sequence encodes a mammalian tetranectin,

69. The use according to claim 67 wherein the nucleotide sequence encodes human or murine tetranectin.

70. The use according to claim 67 wherein the nucleotide sequence encodes a derivative of a native tetranectin wherein up to 10, preferably up to 4, and more preferably 1 or 2, amino acid residues are substituted, deleted or inserted in the α-helices and β-strands and connecting segments of its CTLD.

71. The use according to claim 67 wherein the nucleotide sequence encodes a derivative of human tetranectin, termed htlec, as shown from nucleotide 20 to nucleotide 562 in SEQ ID NO:12.

72. The use according to claim 67 wherein the nucleotide sequence encodes a derivative of human tetranectin, termed htCTLD, as shown from nucleotide 20 to nucleotide 430 in SEQ ID NO:14.

73. The use according to claim 67 wherein the nucleotide sequence encodes a derivative of murine tetranectin, termed

mtlec, as shown from nucleotide 20 to nucleotide 562 in SEQ ID NO:35.

74. The use according to claim 67 wherein the nucleotide sequence encodes a derivative of murine tetranectin, termed mtCTLD, as shown from nucleotide 20 to nucleotide 430 in SEQ ID NO:37.

75. A method of screening a combinatorial library according to any one of claims 20-38 for binding to a specific target which comprises the following steps:

1) expressing a nucleic acids library according to any one of claims 59-61 to display the library of proteins in the display system;
2) contacting the collection of entities displayed with a suitably tagged target substance for which isolation of a CTLD-derived exhibiting affinity for said target substance is desired;
3) harvesting subpopulations of the entities displayed that exhibit affinity for said target substance by means of affinity-based selective extractions, utilizing the tag to which said target substance is conjugated or physically attached or adhering to as a vehicle or means of affinity purification, a procedure commonly referred to in the field as "affinity panning", followed by re-amplification of the sub-library;
4) isolating progressively better binders by repeated rounds of panning and re-amplification until a suitably small number of good candidate binders is obtained; and,
5) if desired, isolating each of the good candidates as an individual clone and subjecting it to ordinary functional and structural characterisation in preparation for final selection of one or more preferred product clones.

76. A method of reformatting a protein according to any one of claims 1-19 or selected from a combinatorial library according to any one of claims 20-38 and containing a CTLD variant exhibiting desired binding properties, in a desired alternative species-compatible framework by excising the nucleic acid fragment encoding the loop region-substituting polypeptide and any required single framework mutations from the nucleic acid encoding said protein using PCR technology, site directed mutagenesis or restriction enzyme digestion and inserting said nucleic acid fragment into the appropriate location(s) in a display- or protein expression vector that harbours a nucleic acid sequence encoding the desired alternative CTLD framework.

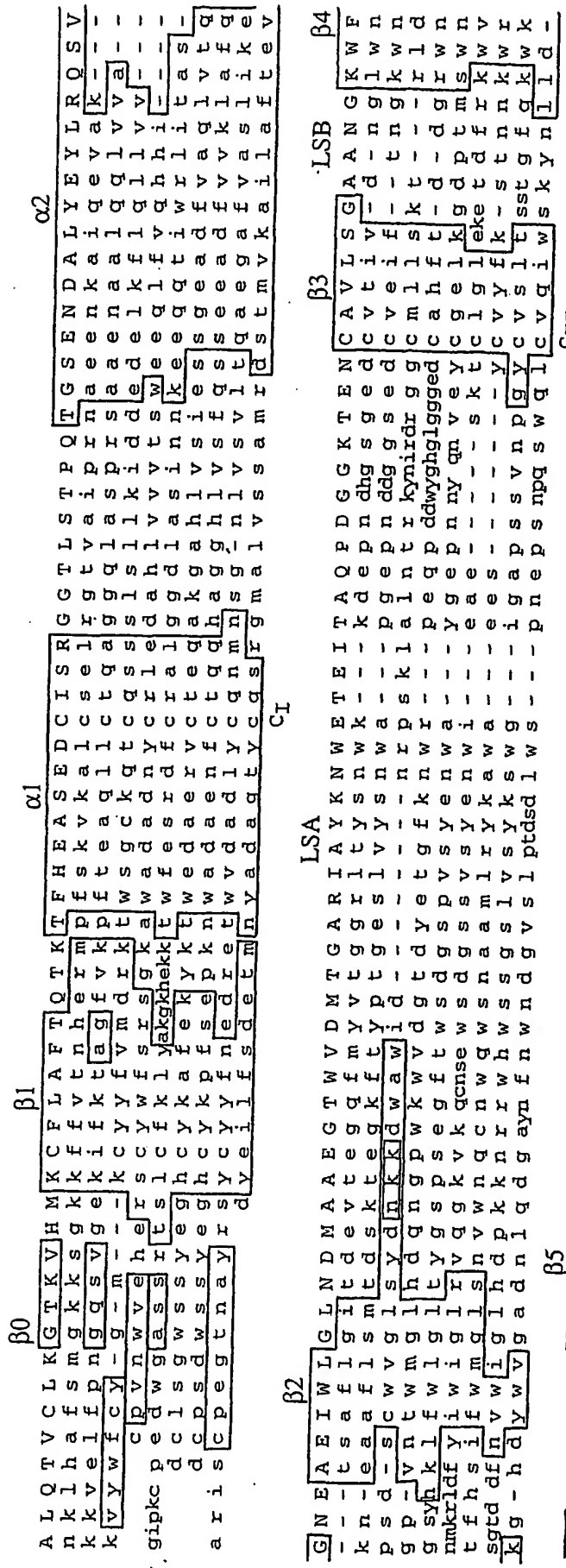


Fig. 1

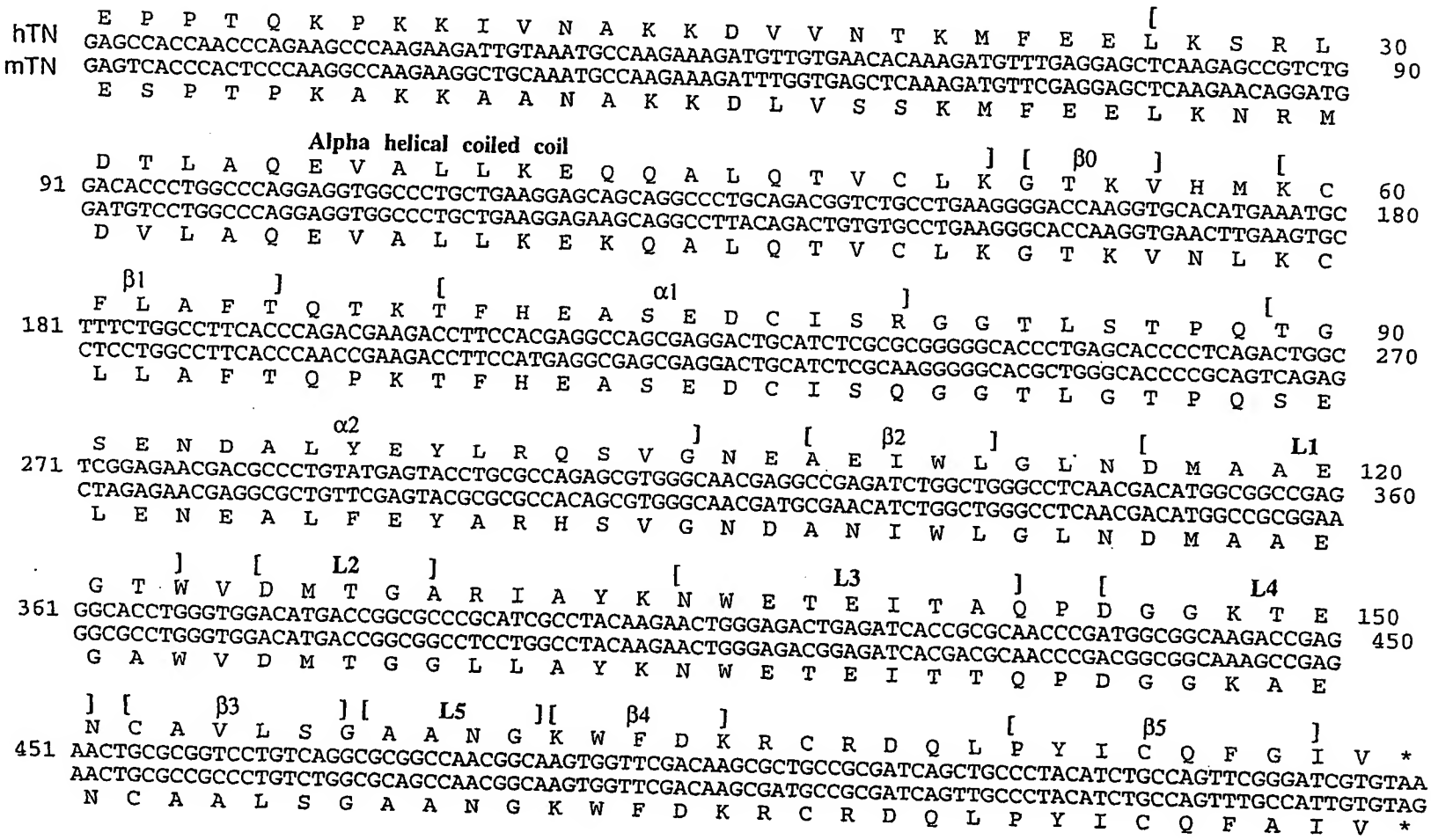
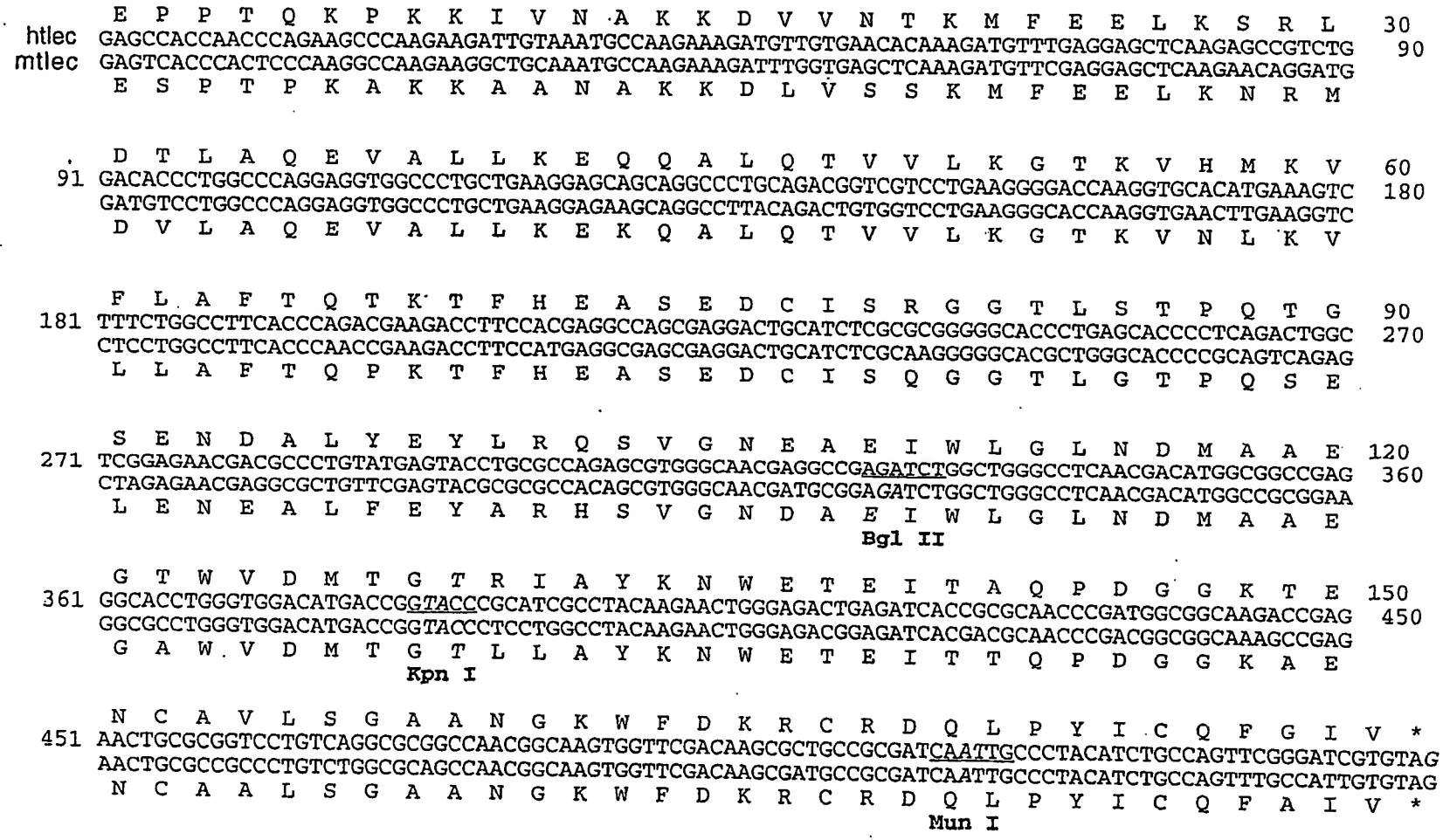

hTN
mTN
Alpha helical coiled coil
β1
α1
α2
β2
L1
L2
L3
L4
β3
L5
β4
β5
β0

Fig. 2


E P P T Q K P K K I V N A K K D V V N T K M F E E L K S R L 30
htlec GAGCCACCAACCCAGAAGCCCAAGAAGATTGTAAATGCCAAGAAAGATGTTGTGAACACAAAGATGTTTGAGGAGCTCAAGAGCCGTCTG 90
mtlec GAGTCACCCACTCCCAAGGCCAAGAAGGCTGCAAATGCCAAGAAAGATTTGGTGAGCTCAAAGATGTTCGAGGAGCTCAAGAACAGGATG
E S P T P K A K K A A N A K K D L V S S K M F E E L K N R M
D T L A Q E V A L L K E Q Q A L Q T V V L K G T K V H M K V 60
91 GACACCCTGGCCCAGGAGGTGGCCCTGCTGAAGGAGCAGCAGGCCCTGCAGACGGTCGTCCTGAAGGGGACCAAGGTGCACATGAAAGTC 180
GATGTCCTGGCCCAGGAGGTGGCCCTGCTGAAGGAGAAGCAGGCCTTACAGACTGTGGTCCTGAAGGGCACCAAGGTGAACTTGAAGGTC
D V L A Q E V A L L K E K Q A L Q T V V L K G T K V N L K V
F L A F T Q T K T F H E A S E D C I S R G G T L S T P Q T G 90
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CTCCTGGCCTTCACCCAACCGAAGACCTTCCATGAGGCGAGCGAGGACTGCATCTCGCAAGGGGGCACGCTGGGCACCCCGCAGTCAGAG
L L A F T Q P K T F H E A S E D C I S Q G G T L G T P Q S E
S E N D A L Y E Y L R Q S V G N E A E I W L G L N D M A A E 120
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L E N E A L F E Y A R H S V G N D A E I W L G L N D M A A E
Bgl II
G T W V D M T G T R I A Y K N W E T E I T A Q P D G G K T E 150
361 GGCACCTGGGTGGACATGACCGGTACCCGCATCGCCTACAAGAACTGGGAGACTGAGATCACCGCGCAACCCGATGGCGGCAAGACCGAG 450
GGCGCCTGGGTGGACATGACCGGTACCCTCCTGGCCTACAAGAACTGGGAGACGGAGATCACGACGCAACCCGACGGCGGCAAAGCCGAG
G A W V D M T G T L L A Y K N W E T E I T T Q P D G G K A E
Kpn I
N C A V L S G A A N G K W F D K R C R D Q L P Y I C Q F G I V *
451 AACTGCGCGGTCCTGTCAGGCGCGGCCAACGGCAAGTGGTTCGACAAGCGCTGCCGCGATCAATTGCCCTACATCTGCCAGTTCGGGATCGTGTAG
AACTGCGCCGCCCTGTCTGGCGCAGCCAACGGCAAGTGGTTCGACAAGCGATGCCGCGATCAATTGCCCTACATCTGCCAGTTTGCCATTGTGTAG
N C A A L S G A A N G K W F D K R C R D Q L P Y I C Q F A I V *
Mun I

Fig. 3

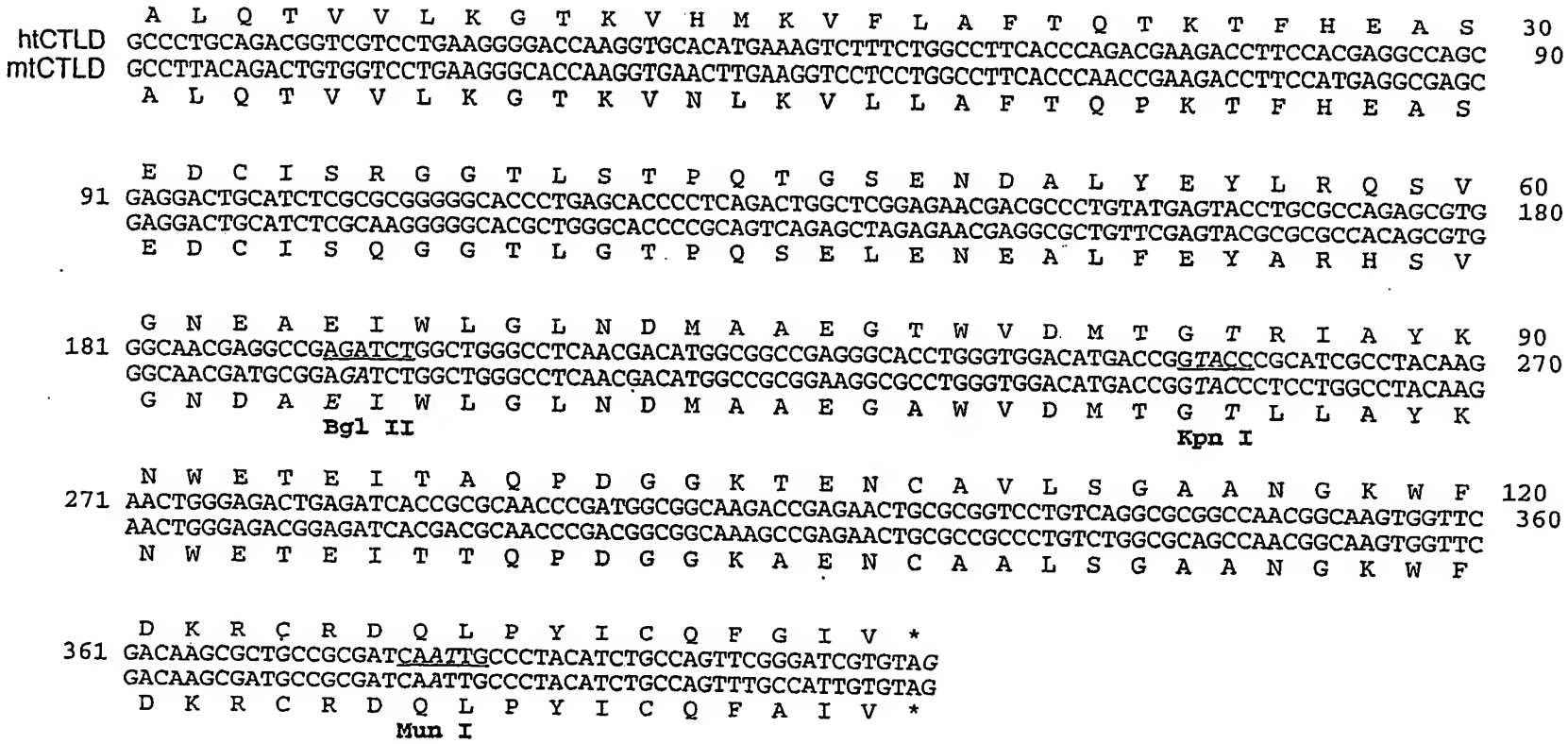
A L Q T V V L K G T K V H M K V F L A F T Q T K T F H E A S 30
htCTLD GCCCTGCAGACGGTCGTCCTGAAGGGGACCAAGGTGCACATGAAAGTCTTTCTGGCCTTCACCCAGACGAAGACCTTCCACGAGGCCAGC 90
mtCTLD GCCTTACAGACTGTGGTCCTGAAGGGCACCAAGGTGAACTTGAAGGTCCTCCTGGCCTTCACCCAACCGAAGACCTTCCATGAGGCGAGC
A L Q T V V L K G T K V N L K V L L A F T Q P K T F H E A S
E D C I S R G G T L S T P Q T G S E N D A L Y E Y L R Q S V 60
91 GAGGACTGCATCTCGCGCGGGGGCACCCTGAGCACCCCTCAGACTGGCTCGGAGAACGACGCCCTGTATGAGTACCTGCGCCAGAGCGTG 180
GAGGACTGCATCTCGCAAGGGGGCACGCTGGGCACCCCGCAGTCAGAGCTAGAGAACGAGGCGCTGTTCGAGTACGCGCGCCACAGCGTG
E D C I S Q G G T L G T P Q S E L E N E A L F E Y A R H S V
G N E A E I W L G L N D M A A E G T W V D M T G T R I A Y K 90
181 GGCAACGAGGCCGAGATCTGGCTGGGCCTCAACGACATGGCGGCCGAGGGCACCTGGGTGGACATGACCGGTACCCGCATCGCCTACAAG 270
GGCAACGATGCGGAGATCTGGCTGGGCCTCAACGACATGGCCGCGGAAGGCGCCTGGGTGGACATGACCGGTACCCTCCTGGCCTACAAG
G N D A E I W L G L N D M A A E G A W V D M T G T L L A Y K
Bgl II
Kpn I
N W E T E I T A Q P D G G K T E N C A V L S G A A N G K W F 120
271 AACTGGGAGACTGAGATCACCGCGCAACCCGATGGCGGCAAGACCGAGAACTGCGCGGTCCTGTCAGGCGCGGCCAACGGCAAGTGGTTC 360
AACTGGGAGACGGAGATCACGACGCAACCCGACGGCGGCAAAGCCGAGAACTGCGCCGCCCTGTCTGGCGCAGCCAACGGCAAGTGGTTC
N W E T E I T T Q P D G G K A E N C A A L S G A A N G K W F
D K R Ç R D Q L P Y I C Q F G I V *
361 GACAAGCGCTGCCGCGATCAATTGCCCTACATCTGCCAGTTCGGGATCGTGTAG
GACAAGCGATGCCGCGATCAATTGCCCTACATCTGCCAGTTTGCCATTGTGTAG
D K R C R D Q L P Y I C Q F A I V *
Mun I

Fig. 4

pT7H6FX-htlec

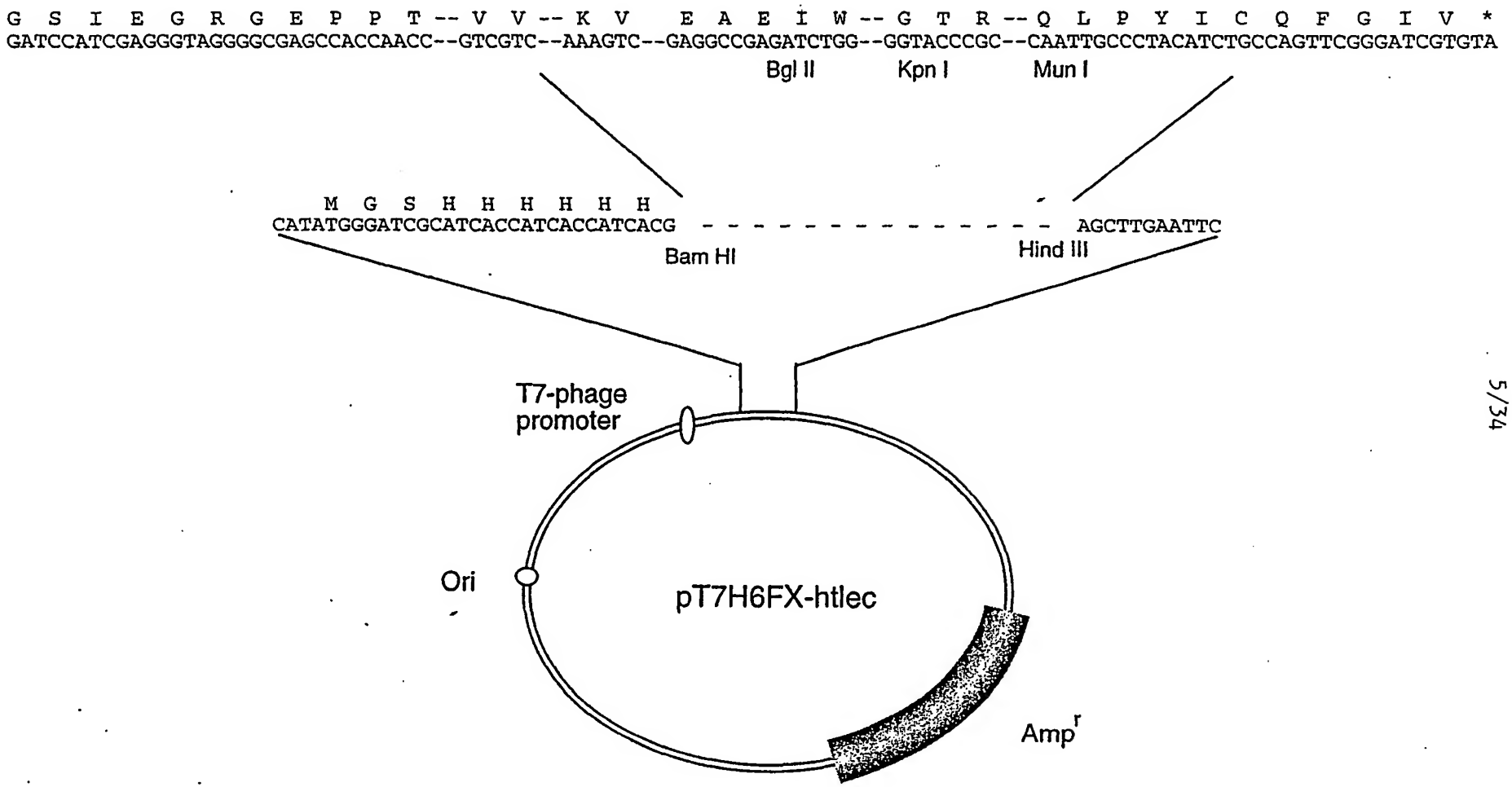

Fig.5

FX-htlec

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 31 E E L K S R L D T L A Q E V A L L K E Q Q A L Q T V V L K G
 61 T K V H M K V F L A F T Q T K T F H E A S E D C I S R G G T
 91 L S T P Q T G S E N D A L Y E Y L R Q S V G N E A E I W L G
121 L N D M A A E G T W V D M T G T R I A Y K N W E T E I T A Q
151 P D G G K T E N C A V L S G A A N G K W F D K R C R D Q L P
181 Y I C Q F G I V
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Fig. 6

pT7H6FX-htCTLD

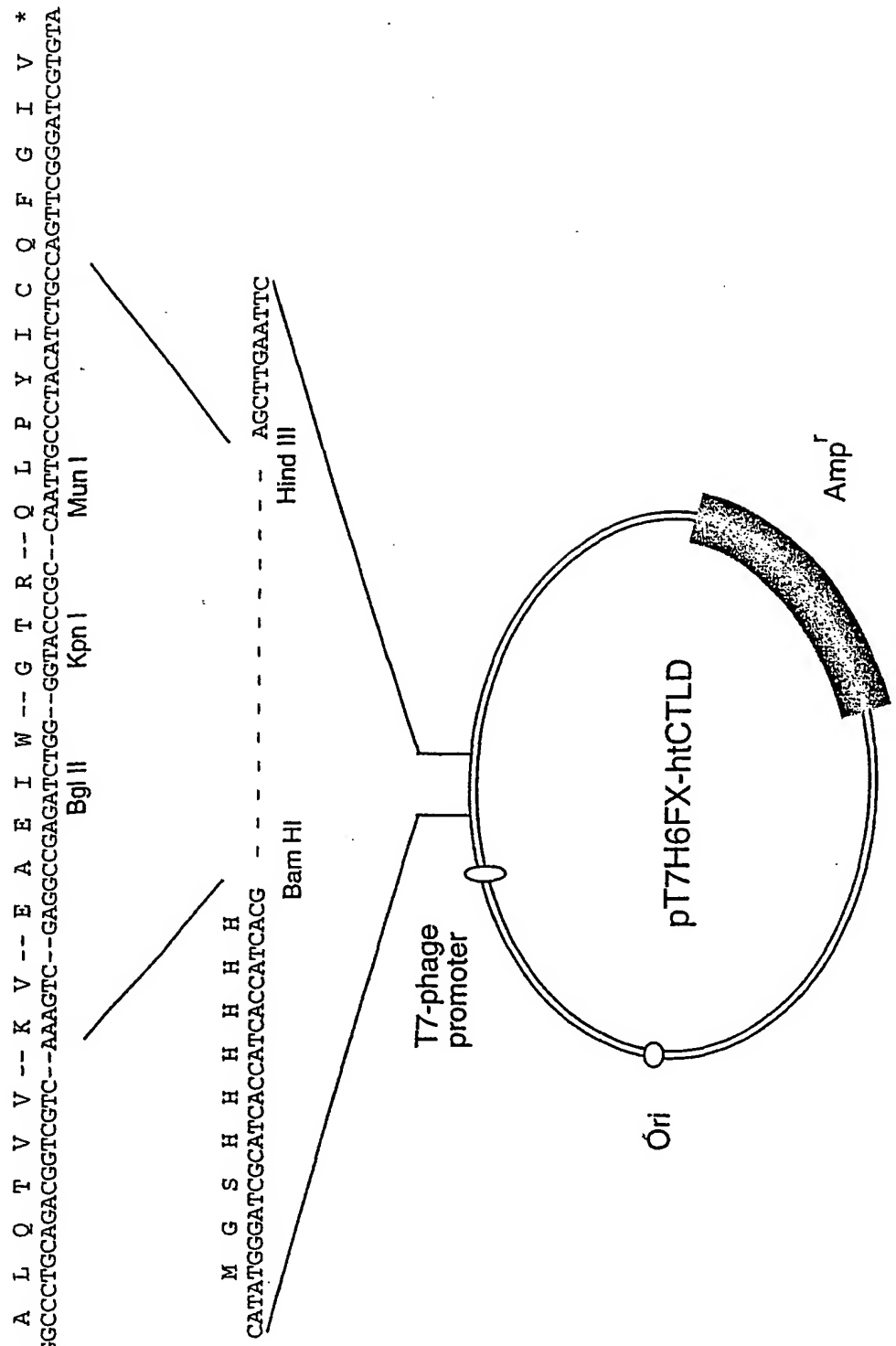
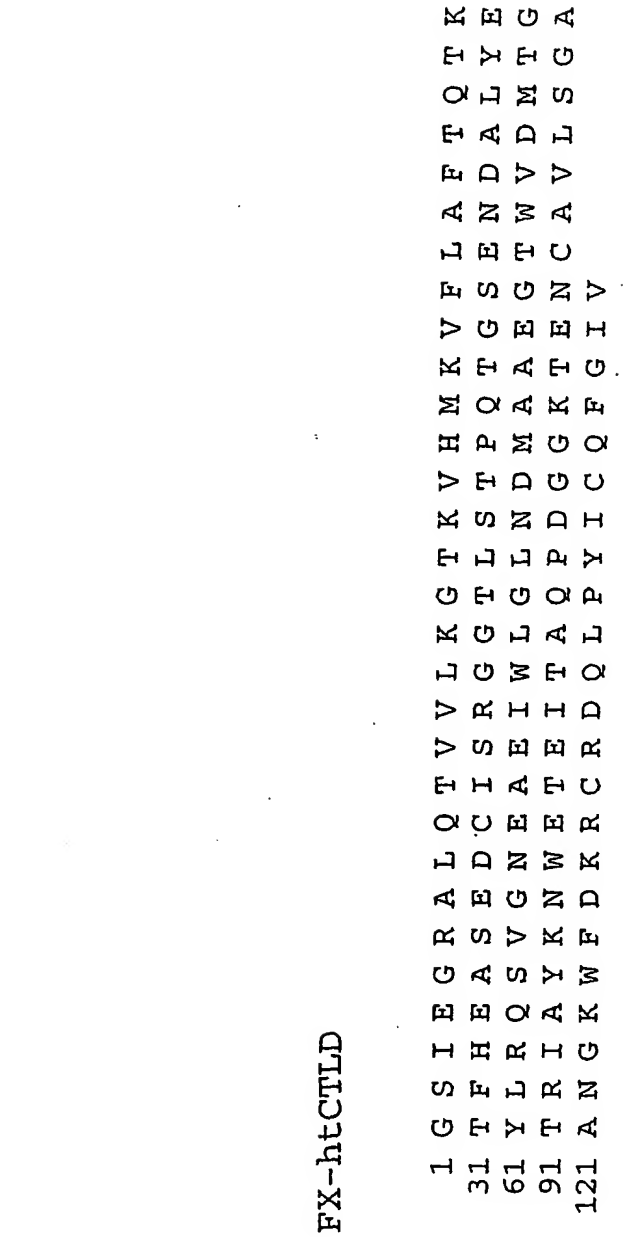


Fig.7


FX-htCTLD
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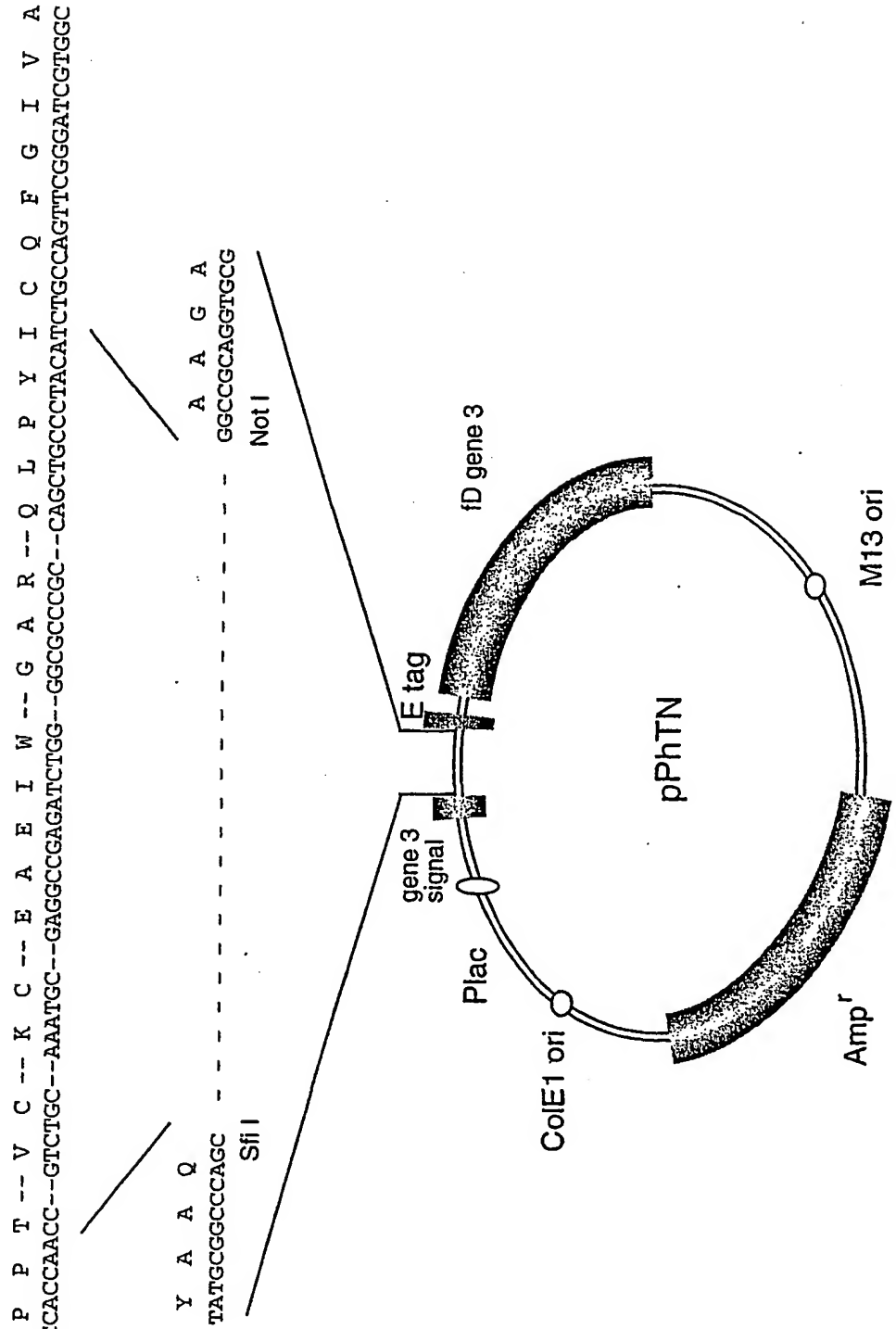
31 T F H E A S E D C I S R G G T L S T P Q T G S E N D A L Y E
61 Y L R Q S V G N E A E I W L G L N D M A A E G T W V D M T G
91 T R I A Y K N W E T E I T A Q P D G G K T E N C A V L S G A
121 A N G K W F D K R C R D Q L P Y I C Q F G I V

Fig.8

pPhTN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Y A A A Q
TATGCGGCCCAGC
Sfi I
A A G A
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Not I
E tag
fD gene 3
M13 ori
gene 3 signal
pPhTN
Plac
ColE1 ori
Amp^r

Fig.9

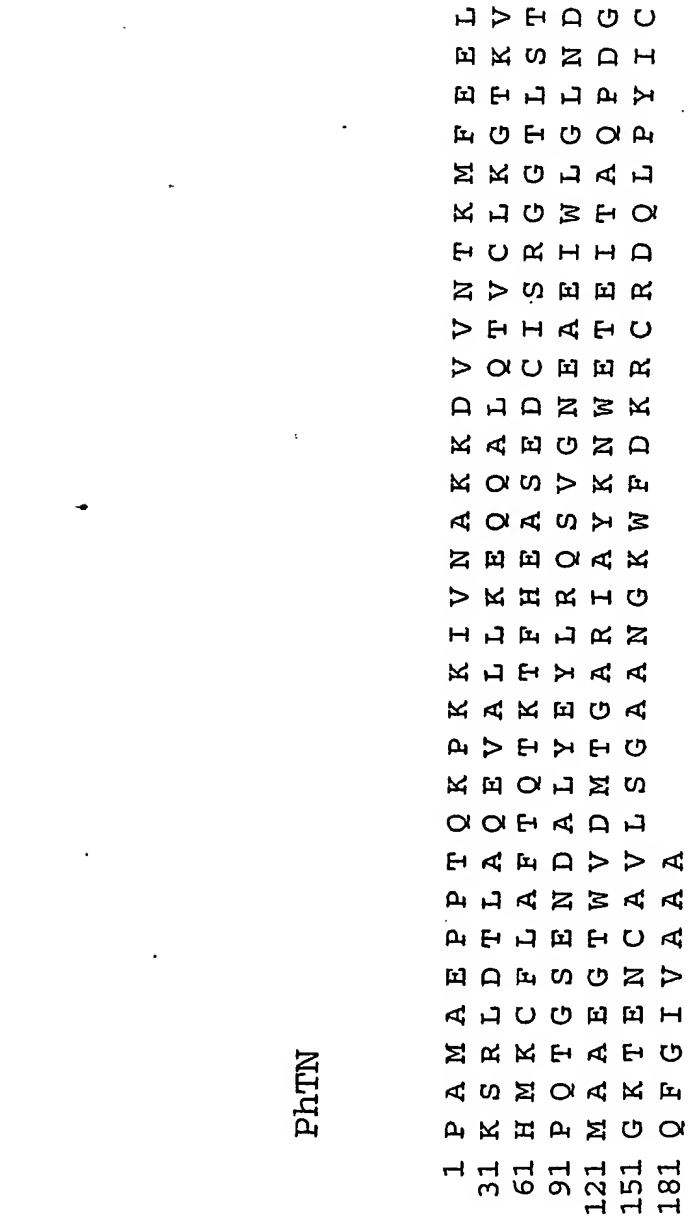
PhTN
1 P A M A E P P T Q K P K K I V N A K K D V V N T K M F E E L
31 K S R L D T L A Q E V A L L K E Q Q A L Q T V C L K G T K V
61 H M K C F L A F T Q T K T F H E A S E D C I S R G G T L S T
91 P Q T G S E N D A L Y E Y L R Q S V G N E A E I W L G L N D
121 M A A E G T W V D M T G A R I A Y K N W E T E I T A Q P D G
151 G K T E N C A V L S G A A N G K W F D K R C R D Q L P Y I C
181 Q F G I V A A A

Fig.10

pPhTN3

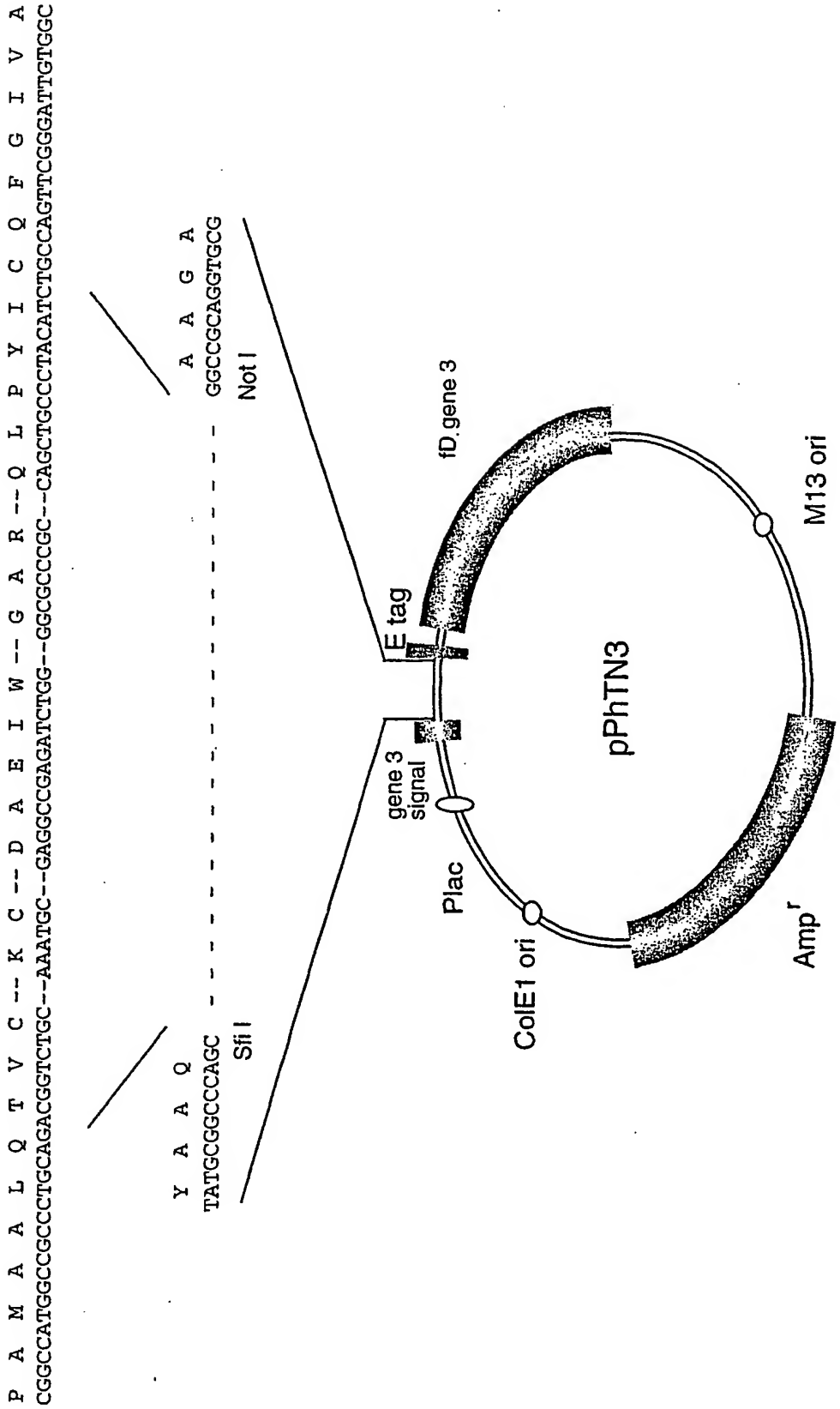

Fig.11

PhTN3

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 31 H E A S E D C I S R G G T L S T P Q T G S E N D A L Y E Y L
 61 R Q S V G N E A E I W L G L N D M A A E G T W V D M T G A R
 91 I A Y K N W E T E I T A Q P D G G K T E N C A V L S G A A N
121 G K W F D K R C R D Q L P Y I C Q F G I V A A A
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Fig.12

pPhtlec

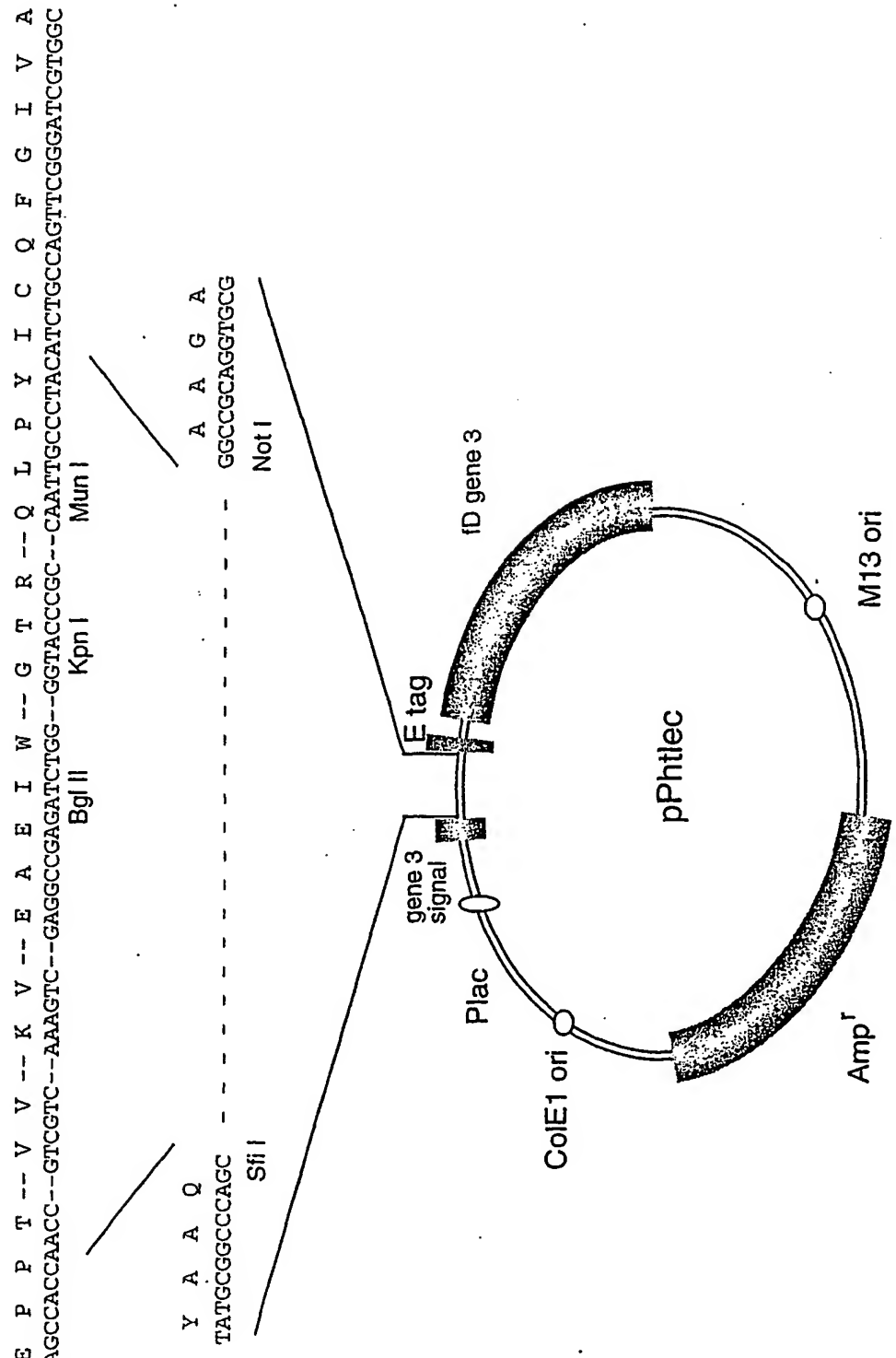
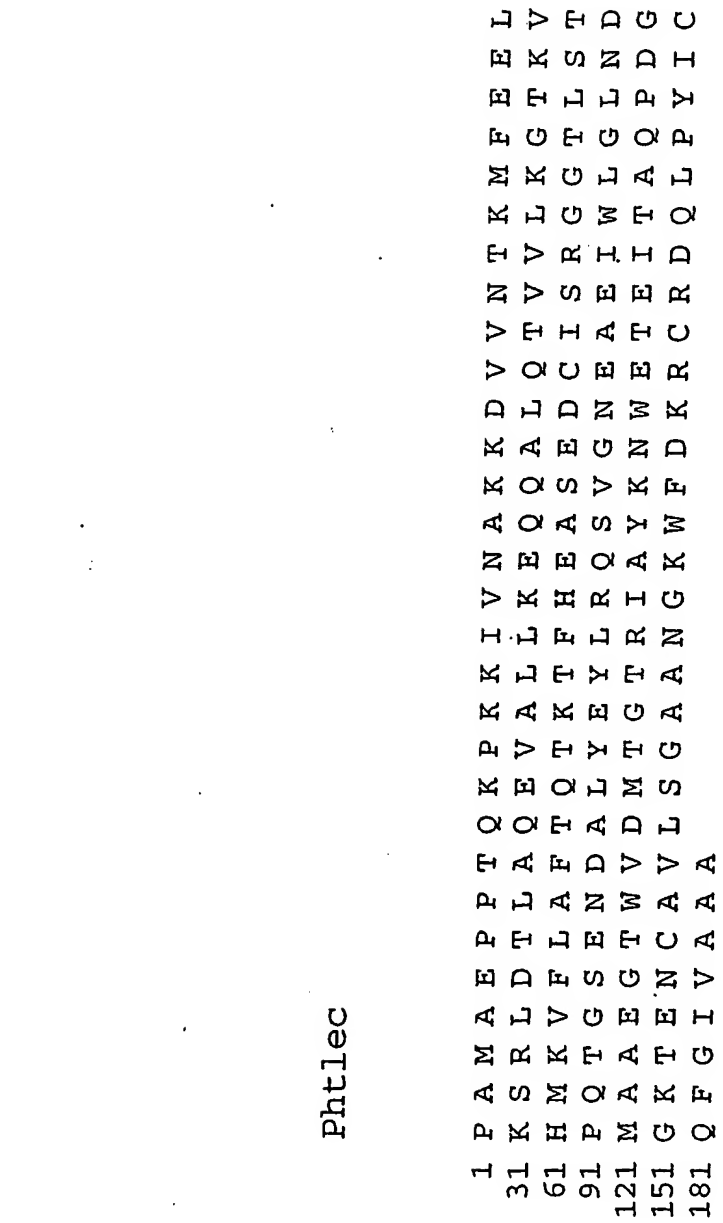


Fig.13


Phtlec
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31 K S R L D T L A Q E V A L L K E Q Q A L Q C I S V N E E K
61 H M K V F L A F T Q T K F H E A S E D C E A E I W L G L N
91 P Q T G S E N D A L Y E Y L R Q S V G N E T E I L T A P D
121 M A A E G T W V D M T G T R I A Y K N W E C R E A Q L Y
151 G K T E N C A V L S G A A N G K W F D K R D L Q L P I C
181 Q F G I V A A A

Fig.14

pPhtCTLD

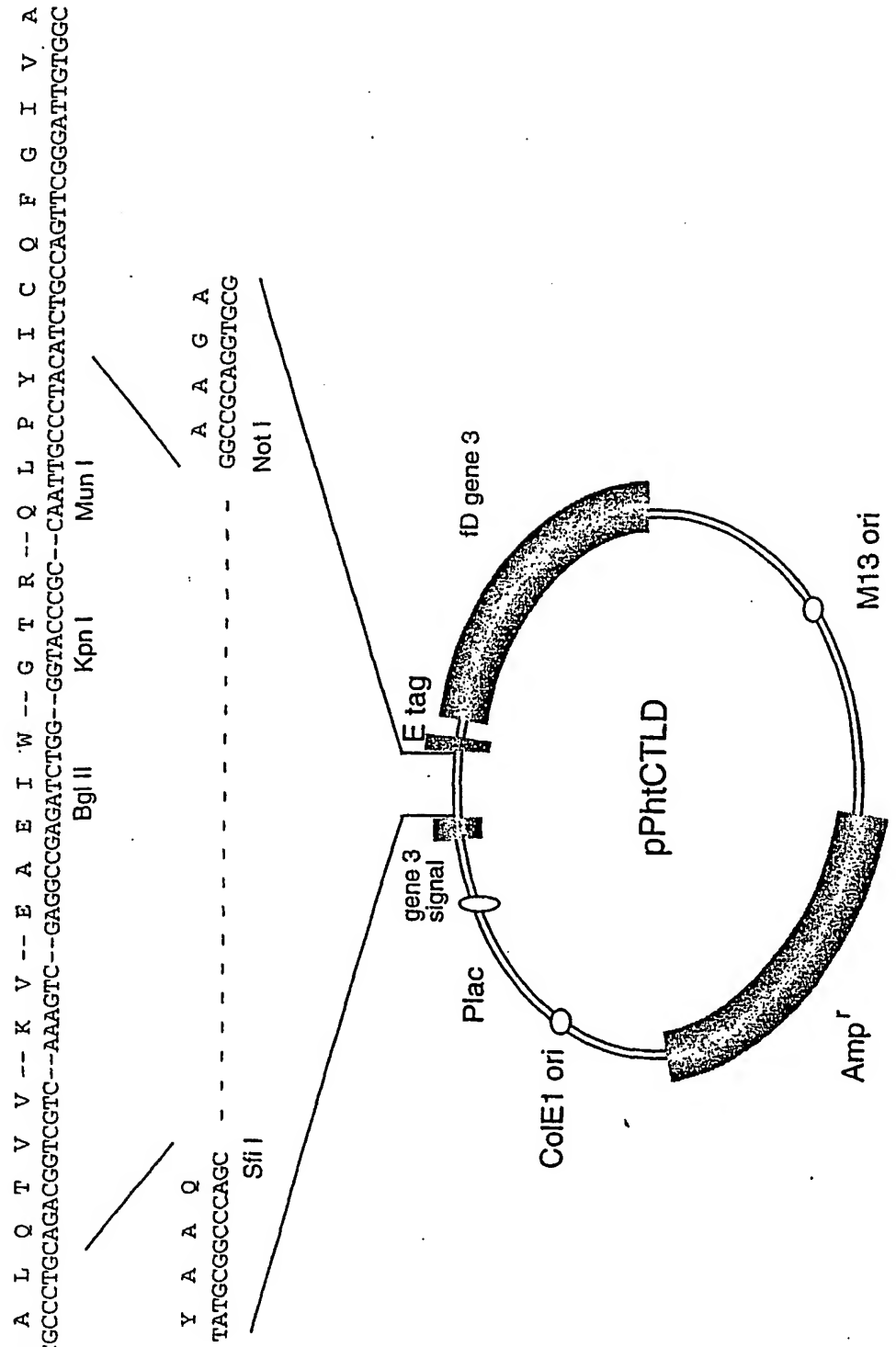

Fig.15

PhtCTLD

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  1 P A M A A L Q T V V L K G T K V H M K V F L A F T Q T K T F
 31 H E A S E D C I S R G G T L S T P Q T G S E N D A L Y E Y L
 61 R Q S V G N E A E I W L G L N D M A A E G T W V D M T G T R
 91 I A Y K N W E T E I T A Q P D G G K T E N C A V L S G A A N
121 G K W F D K R C R D Q L P Y I C Q F G I V A A A
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Fig.16

pUC-mtlec

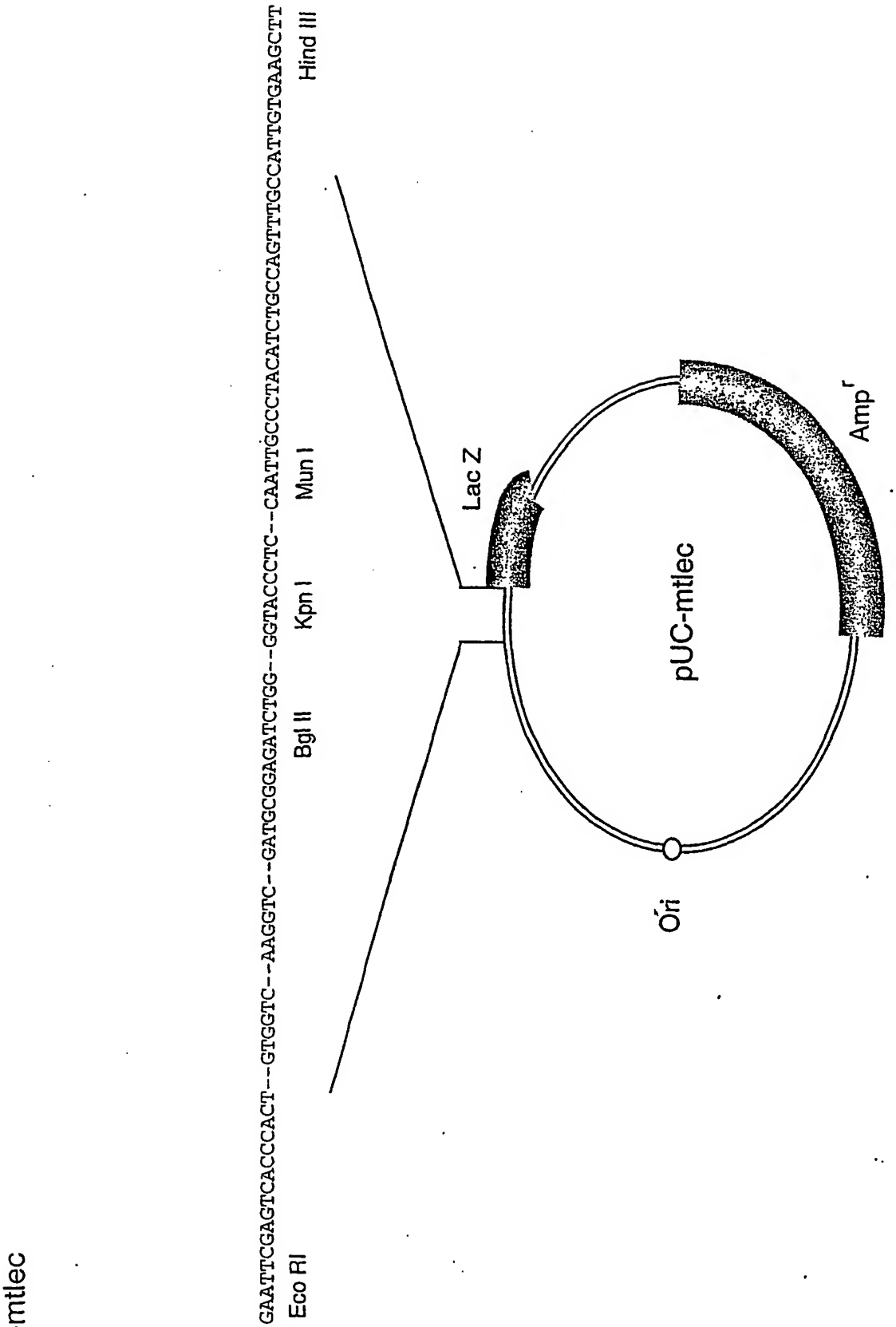


Fig.17

pT7H6FX-mtlec

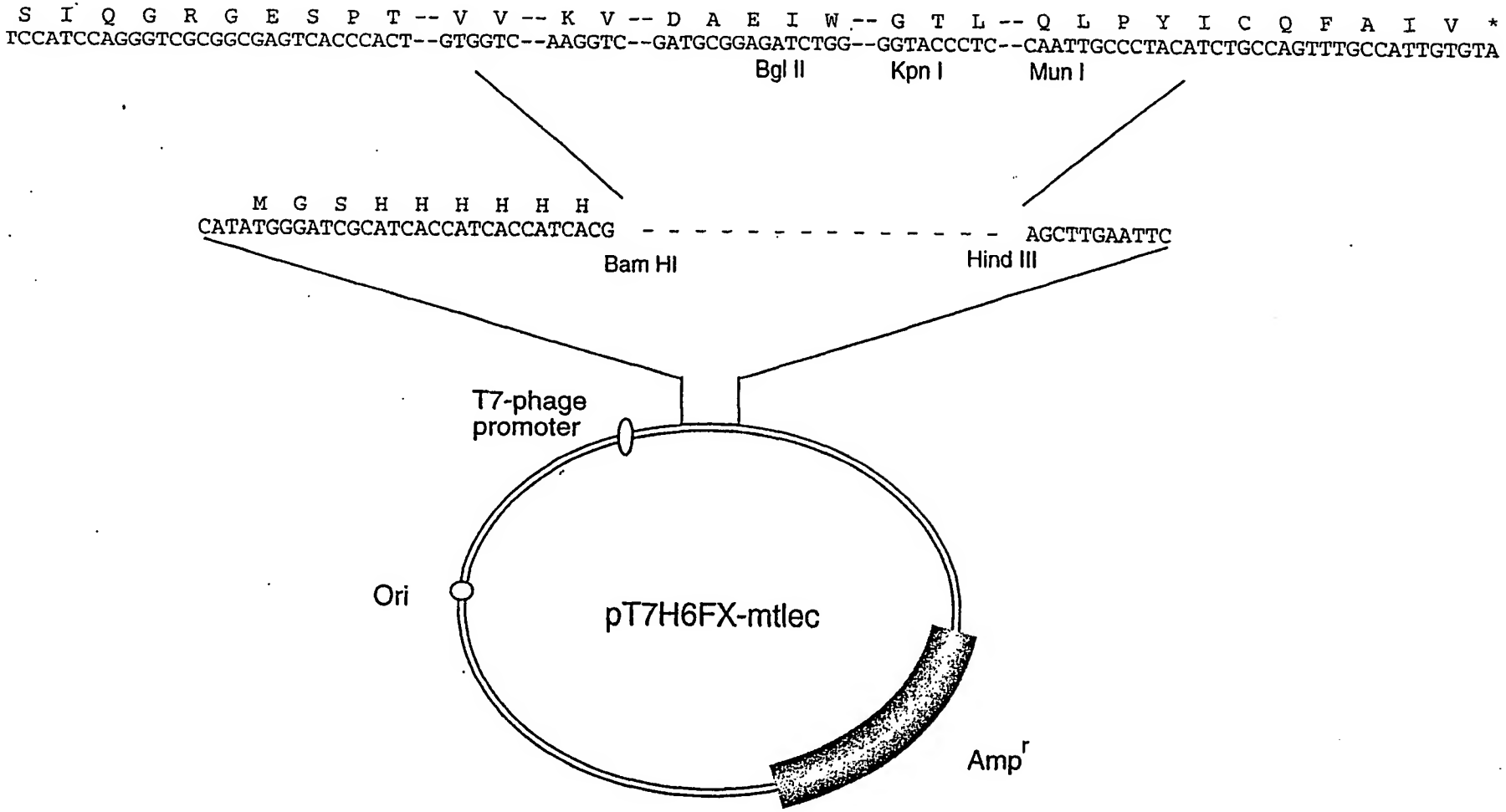
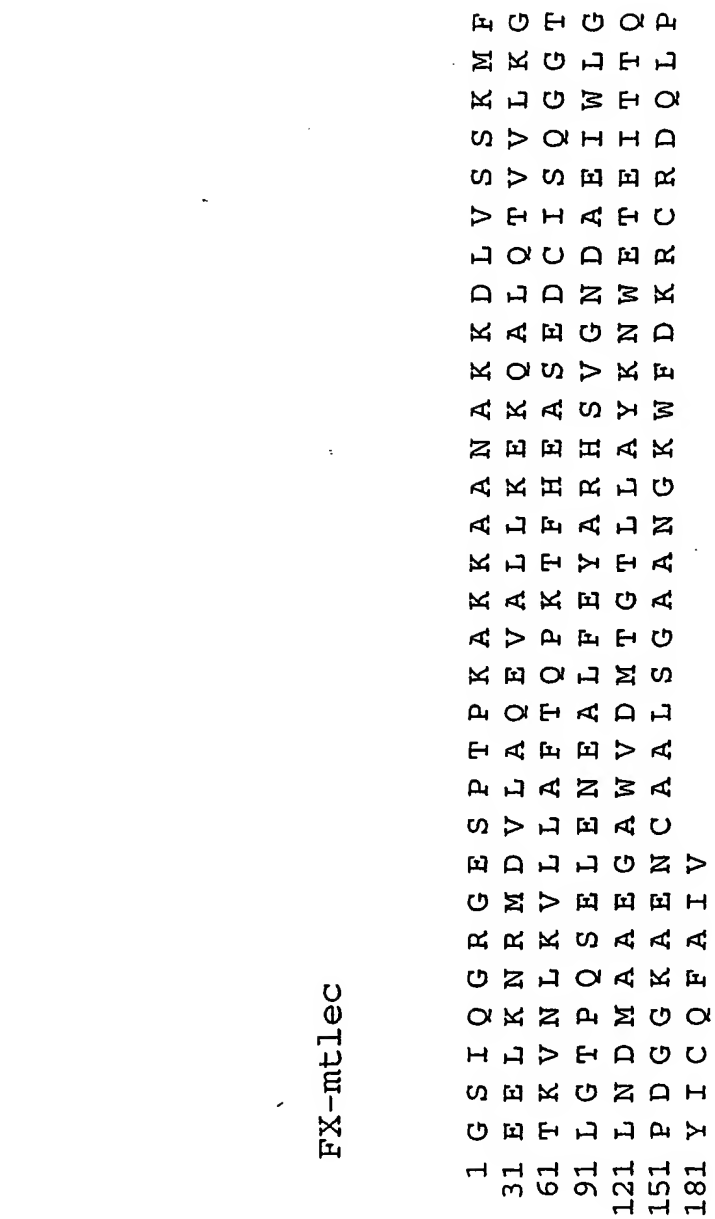


Fig.18


FX-mtlec
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31 E E L K N R M D V L A Q E V A L L K E K Q A L Q T V V L K G
61 T K V N L K V L L A F T Q P K T F H E A S E D C I S Q G G T
91 L G T P Q S E L E N E A L F E Y A R H S V G N D A E I W L G
121 L N D M A A E G A W V D M T G T L L A Y K N W E T E I T T Q
151 P D G G K A E N C A A L S G A A N G K W F D K R C R D Q L P
181 Y I C Q F A I V

Fig.19

pT7H6FX-mtCTLD

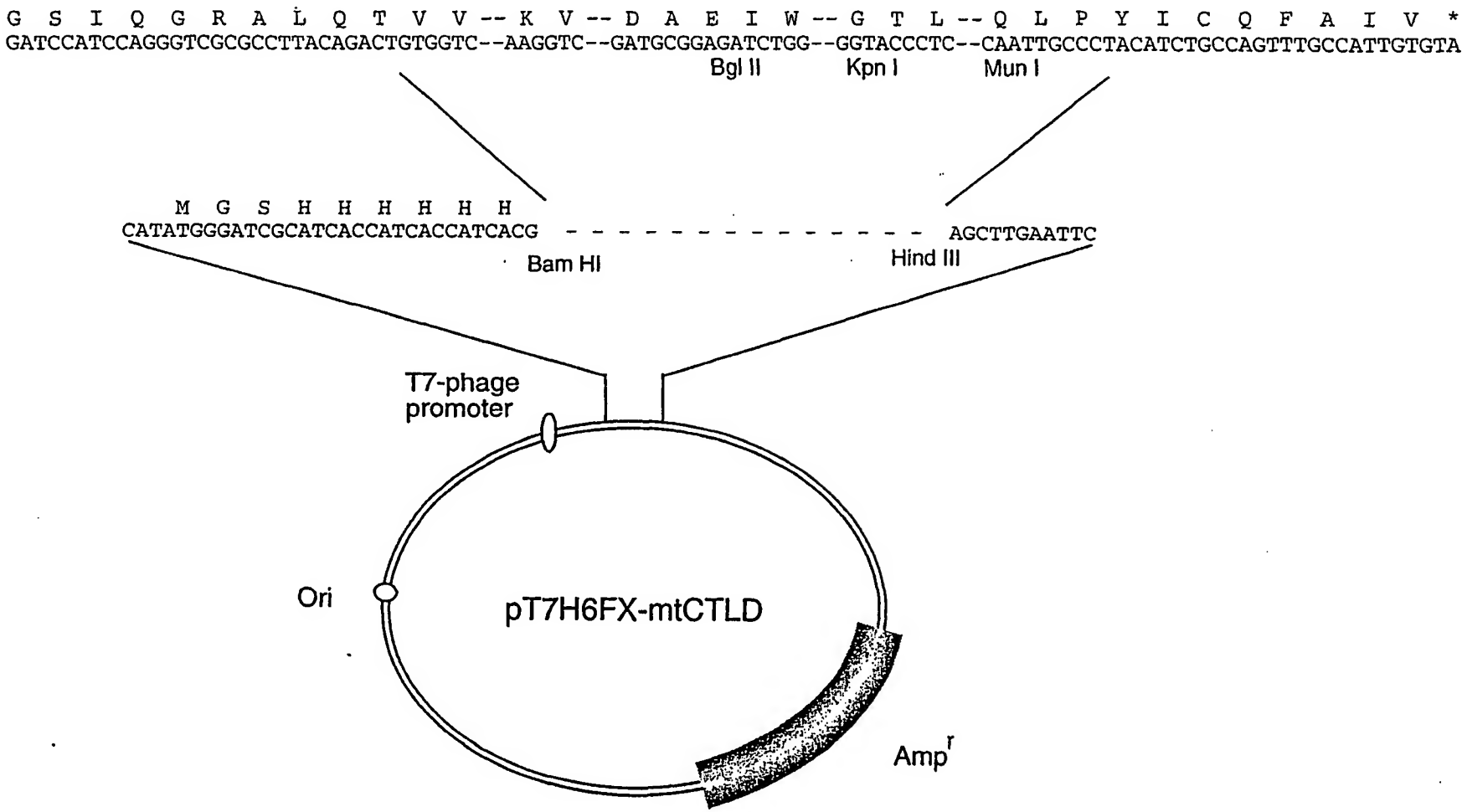
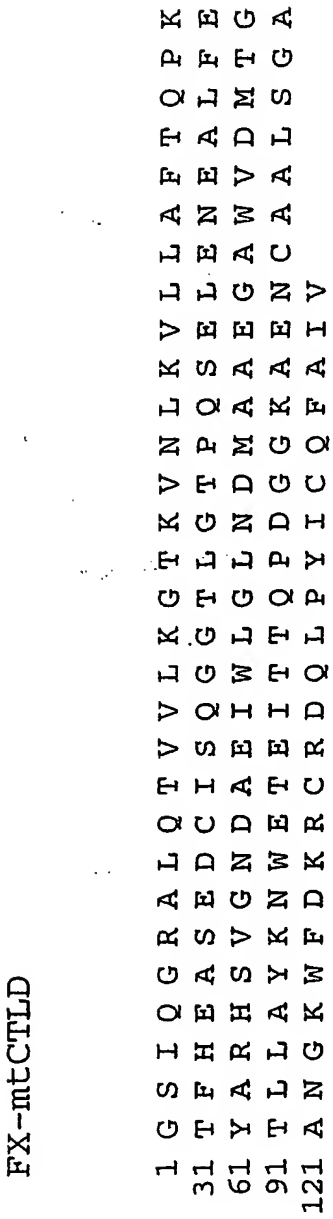


Fig.20


FX-mtCTLD
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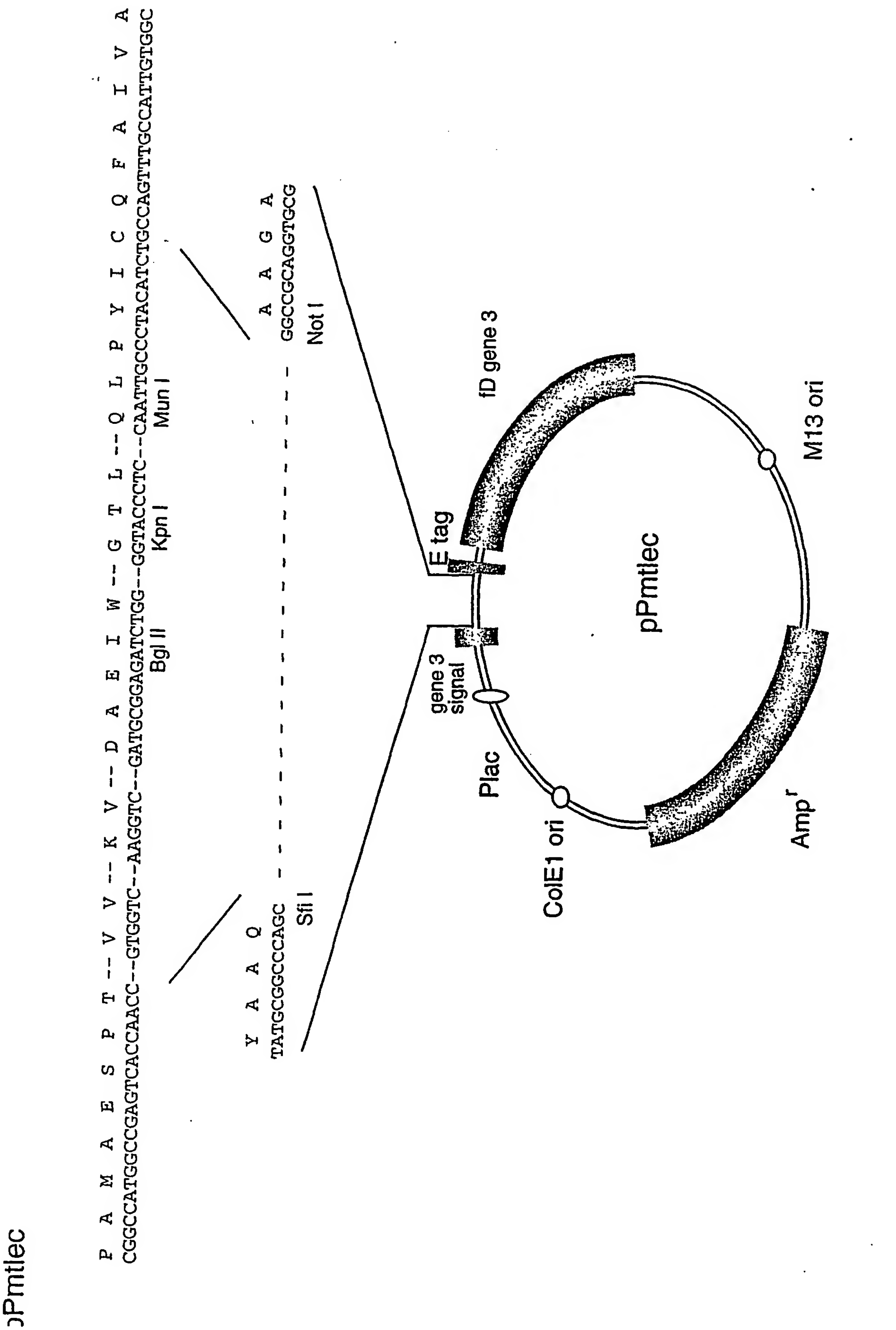
31 T F H E A S E D C I S Q G G T L G T P Q S E L E N E A L F E
61 Y A R H S V G N D A E I W L G L N D M A A E G A W V D M T G
91 T L L A Y K N W E T E I T T Q P D G G K A E N C A A L S G A
121 A N G K W F D K R C R D Q L P Y I C Q F A I V

Fig.21


pPmtlec
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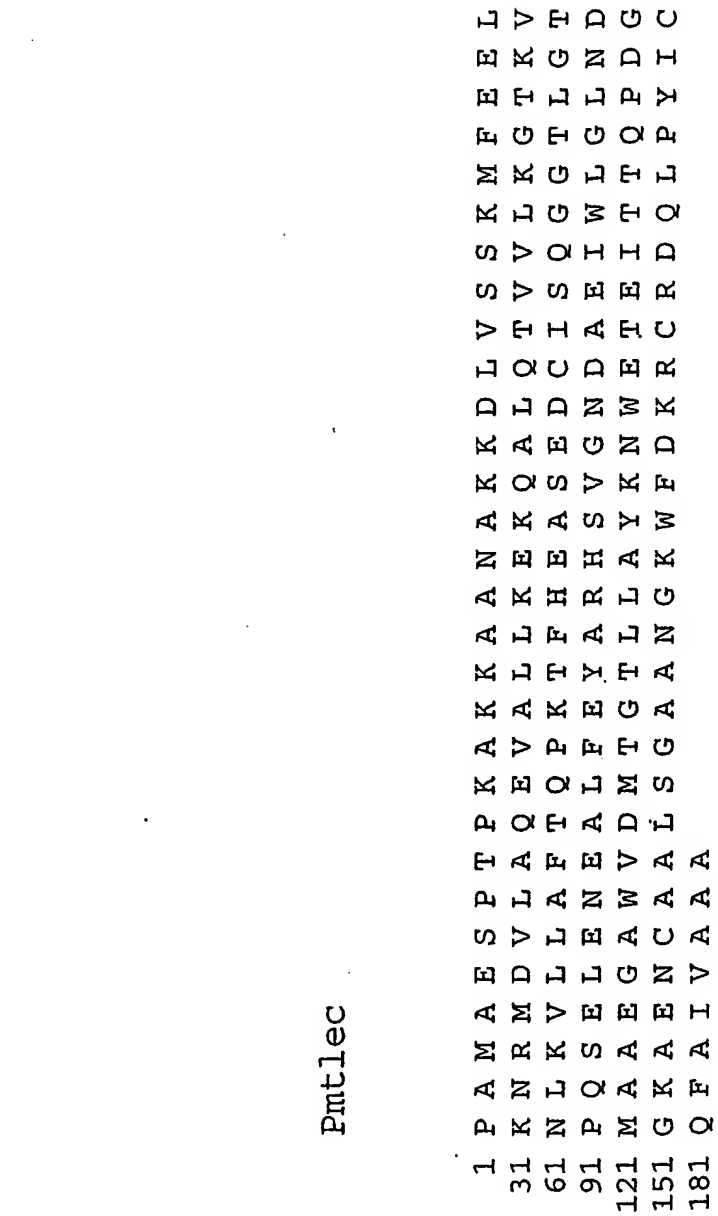
Bgl II
Kpn I
Mun I
Y A A Q
TATGCGGCCCAGC
Sfi I
A A G A
GGCCGCAGGTGCG
Not I
fD gene 3
E tag
gene 3 signal
Plac
ColE1 ori
Amp^r
M13 ori
pPmtlec

Fig.22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31 K N R M D V L A F T Q E V A L L K E K Q A L Q T V V L K G T L G T K V
61 N L K V L L A F T Q P K T F H E A S E D C I S Q G G T L G L N D
91 P Q S E L E N E A L F E Y A R H S V G N D A E I W L G L P D G
121 M A A E G A W V D M T G T L L A Y K N W E T E I T T Q L P Y I C
151 G K A E N C A A L S G A A N G K W F D K R C R D D Q Q L P Y I C
181 Q F A I V A A A A

Fig.23

pPmtCTLD

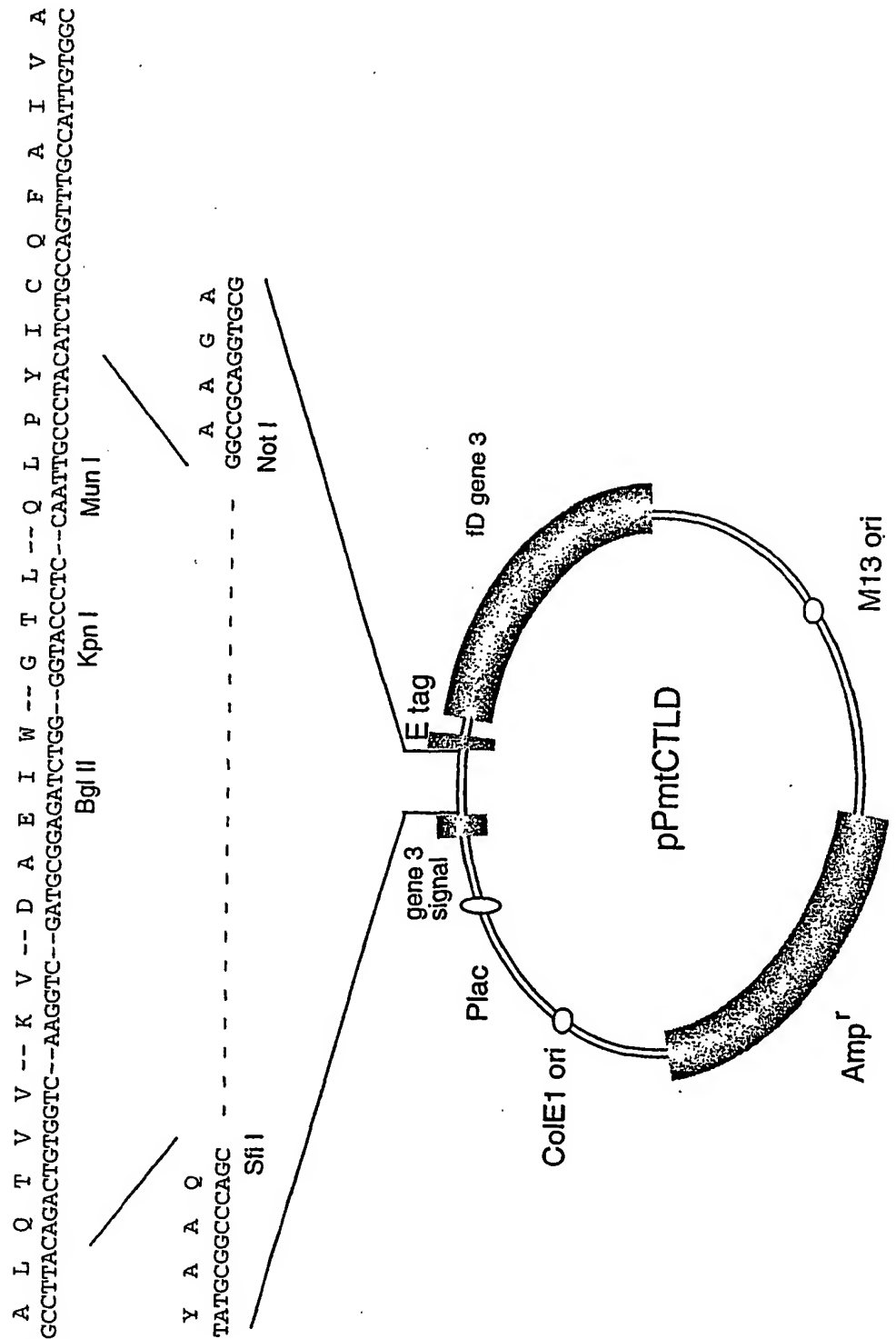

Fig.24

PmtCTLD

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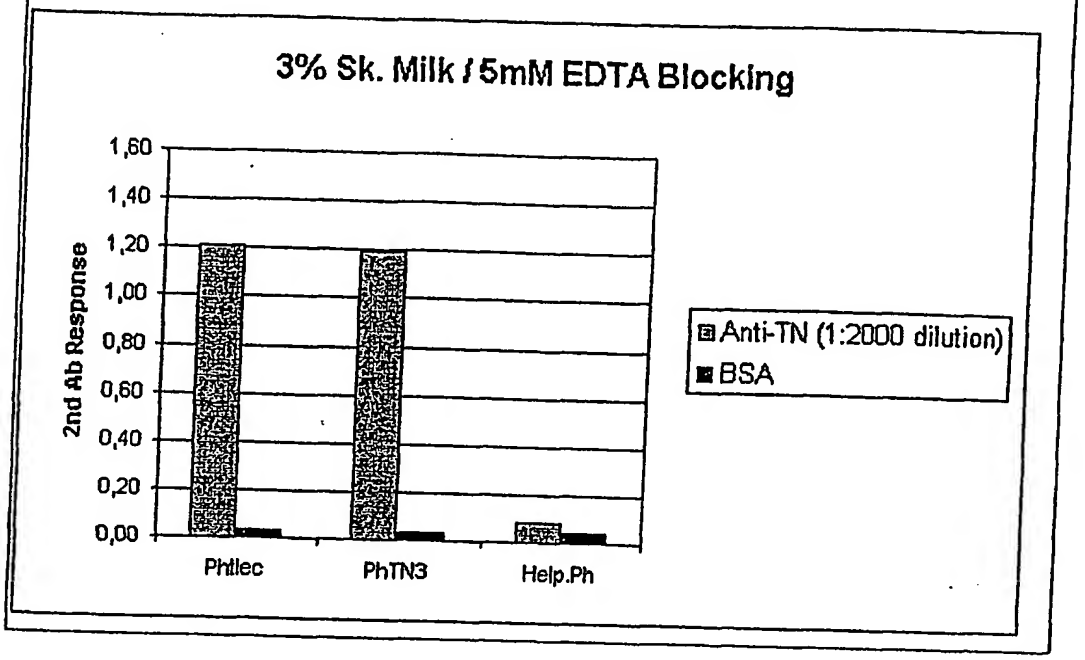
 31 H E A S E D C I S Q G G T L G T P Q S E L E N E A L F E Y A
 61 R H S V G N D A E I W L G L N D M A A E G A W V D M T G T L
 91 L A Y K N W E T E I T T Q P D G G K A E E N C A A L S G A A N
121 G K W F D K R C R D Q L P Y I C Q F A I V A A A A
```

Fig.25

Figure 26

(Panel A)
Anti-TN (1:2000 dilution)
BSA

	Phtlec	PhTN3	Help.Ph
	1,214	1,197	0,084
	0,037	0,035	0,038



(Panel B)
Anti-TN (1:2000 dilution)
BSA

	Phtlec	PhTN3	Help.Ph
	1,192	1,367	0,049
	0,036	0,038	0,039

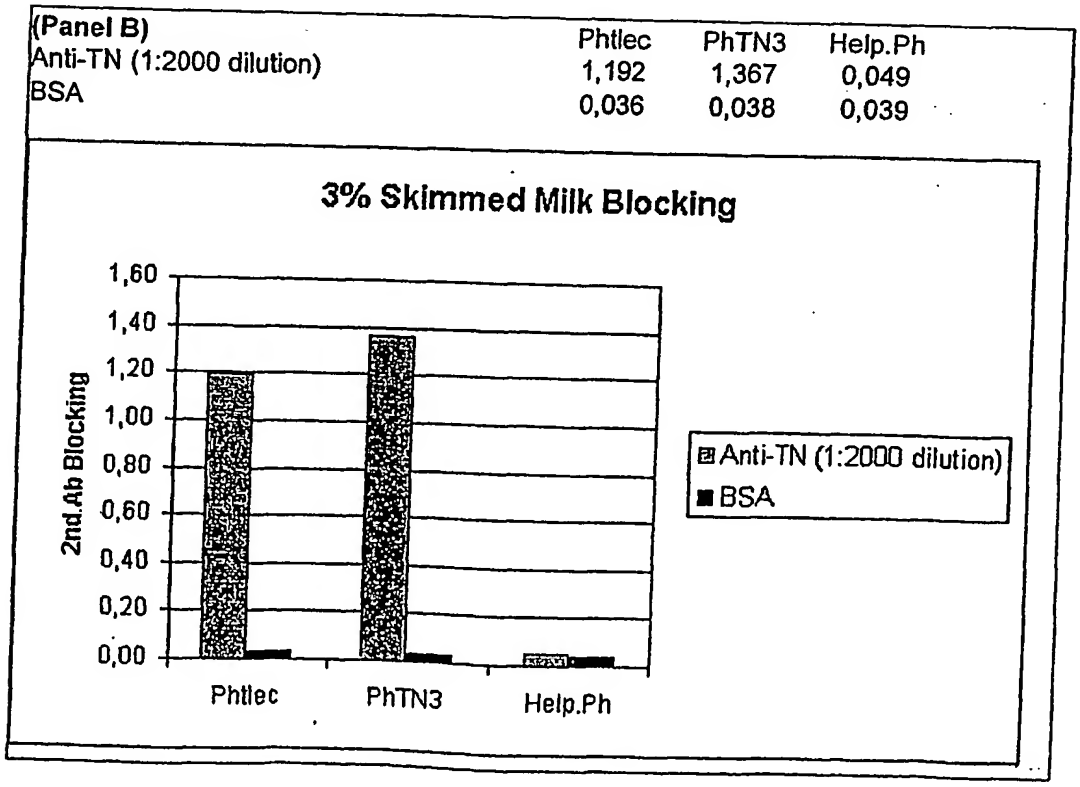
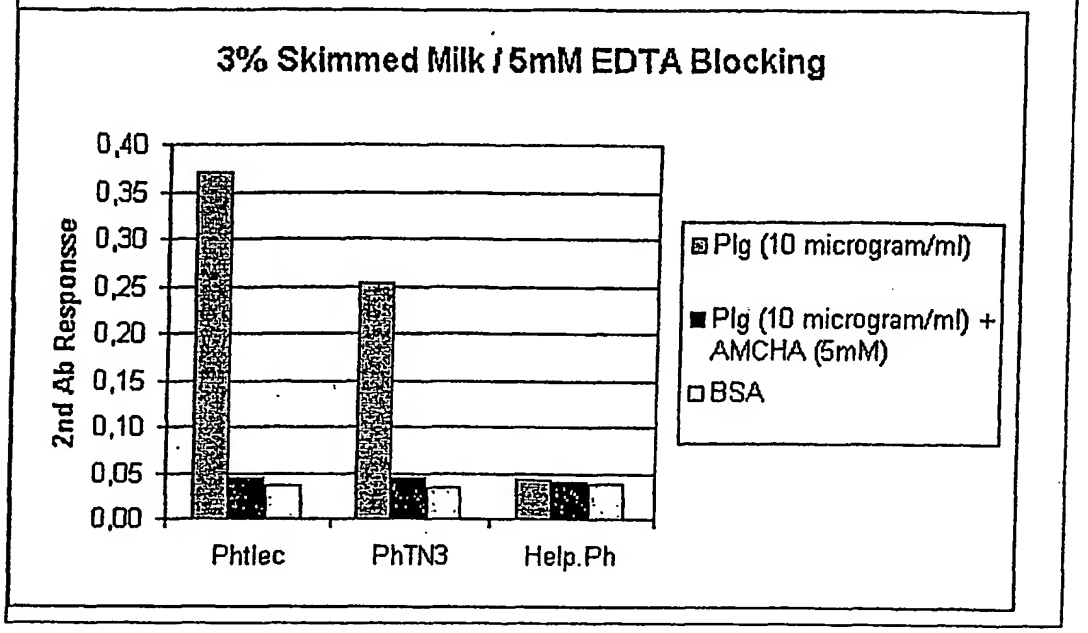


Figure 27

(Panel A)	Phtlec	PhTN3	Help.Ph
Plg (10 microgram/ml)	0,370	0,253	0,042
Plg (10 microgram/ml) + AMCHA (5mM)	0,044	0,044	0,040
BSA	0,037	0,035	0,038



(Panel B)	Phtlec	PhTN3	Help.Ph
Plg (10 microgram/ml)	0,303	0,159	0,040
Plg (10 microgram/ml) + AMCHA (5mM)	0,040	0,049	0,040
BSA	0,036	0,038	0,039

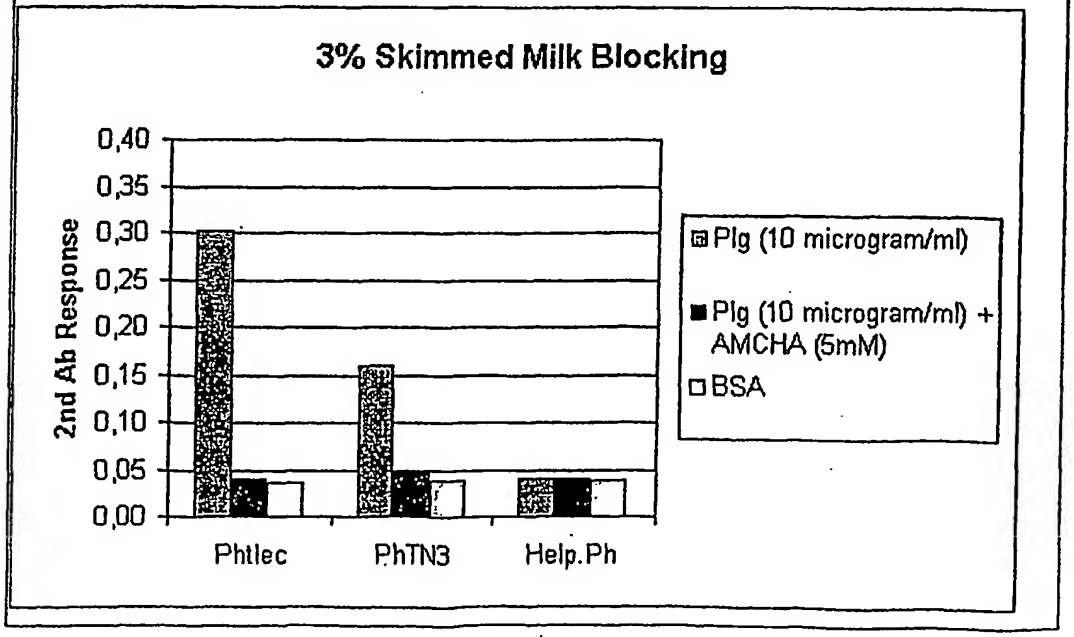


Figure 28

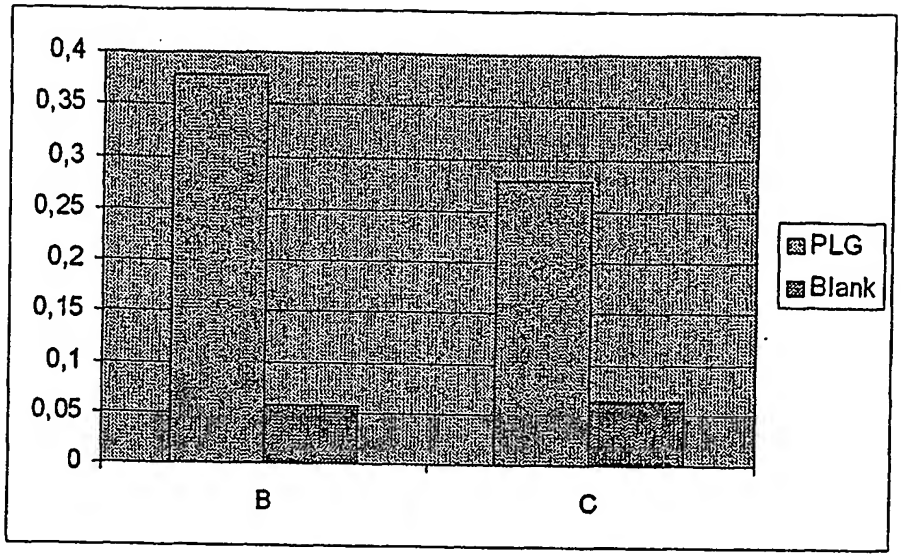
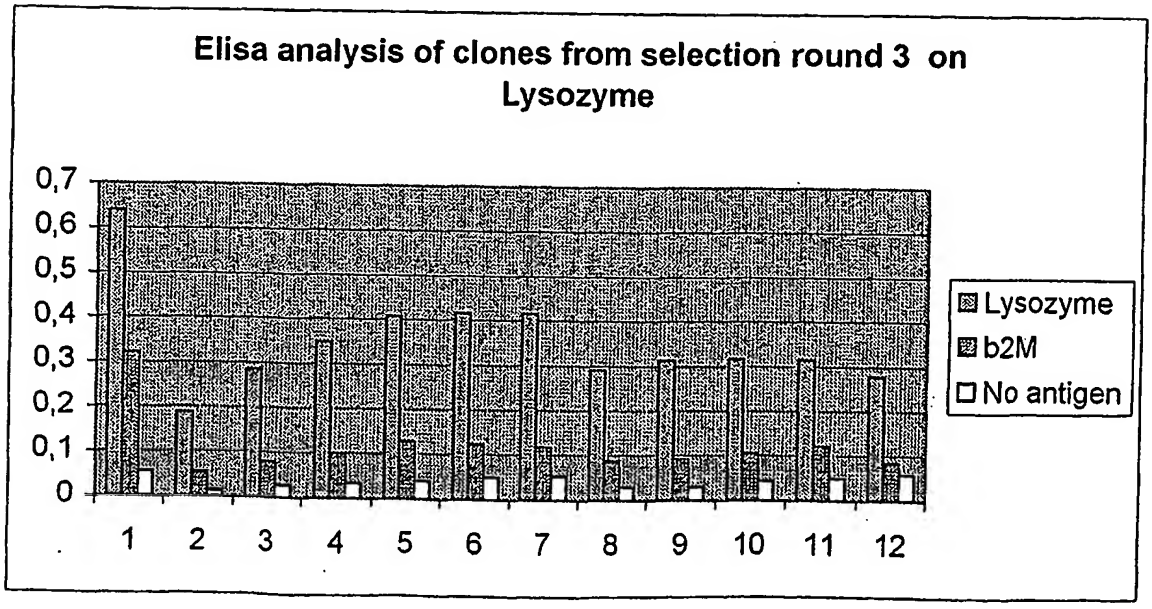
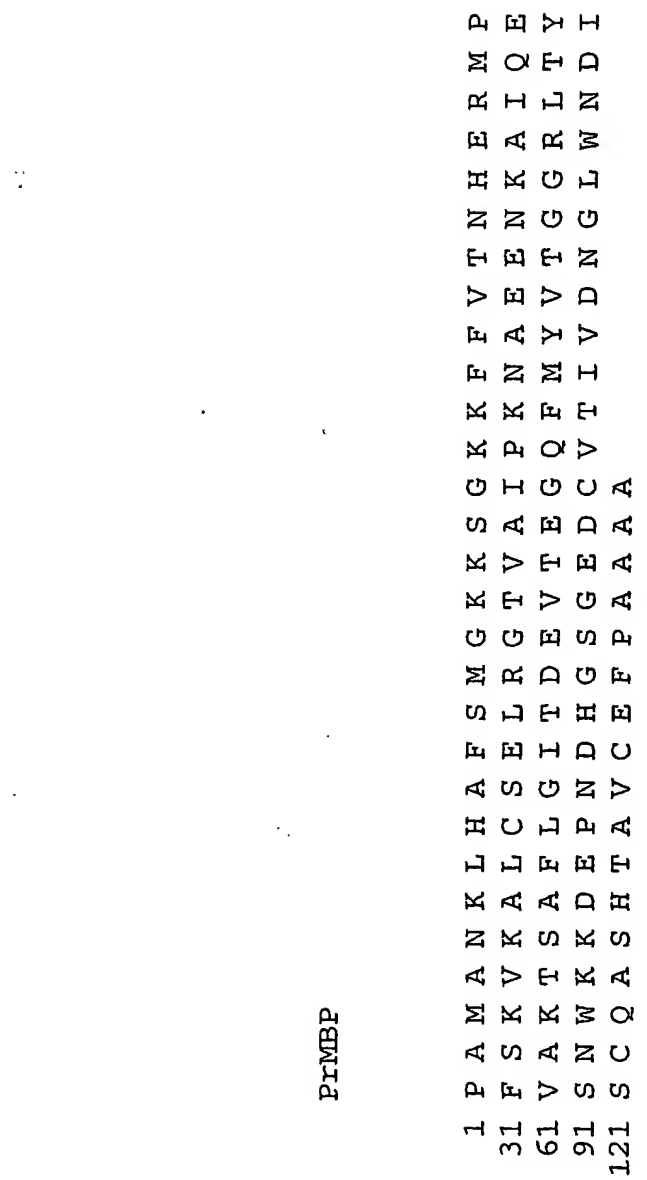


Figure 29




PrMBP
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31 F S K V K A L C S E L R G T V A I P K N A E E N K A I Q E
61 V A K T S A F L G I T D E V T E G Q F M Y V T G G R L T Y
91 S N W K K D E P N D H G S G E D C V T I V D N G L W N D I
121 S C Q A S H T A V C E F P A A A A

Fig.30

pPrMBP

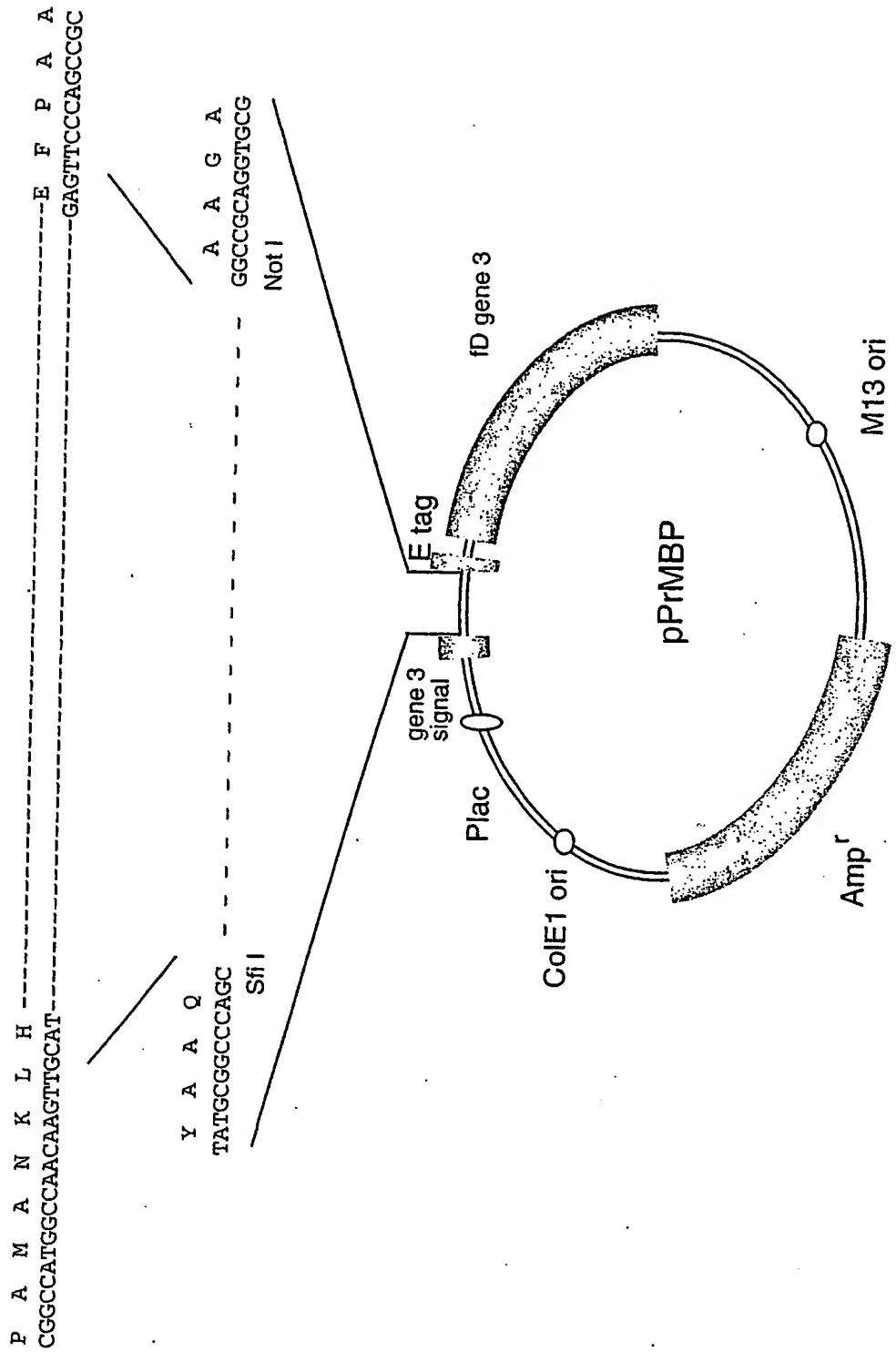
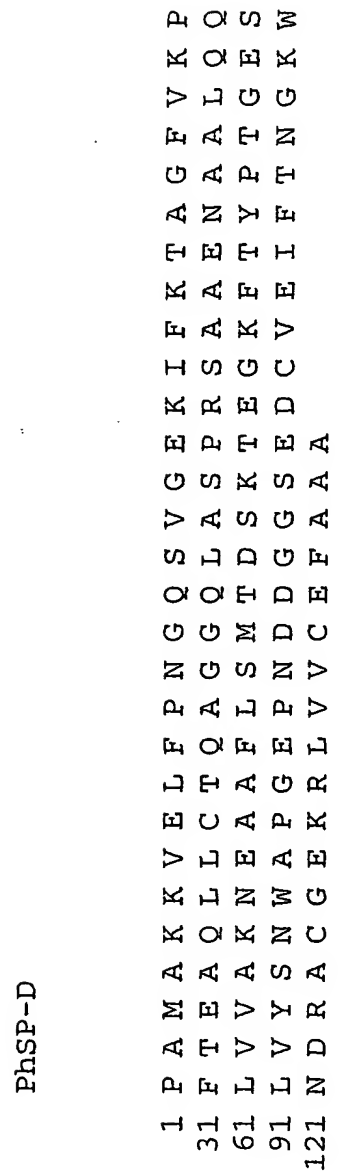


Fig.31


PhSP-D
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61 L V V A K N E A A F L S M T D S K T E G K F T Y P T G E S
91 L V Y S N W A P G E P N D D G G S E D C V E I F T N G K W
121 N D R A C G E K R L V V C E F A A A

Fig. 32

pPhSP-D

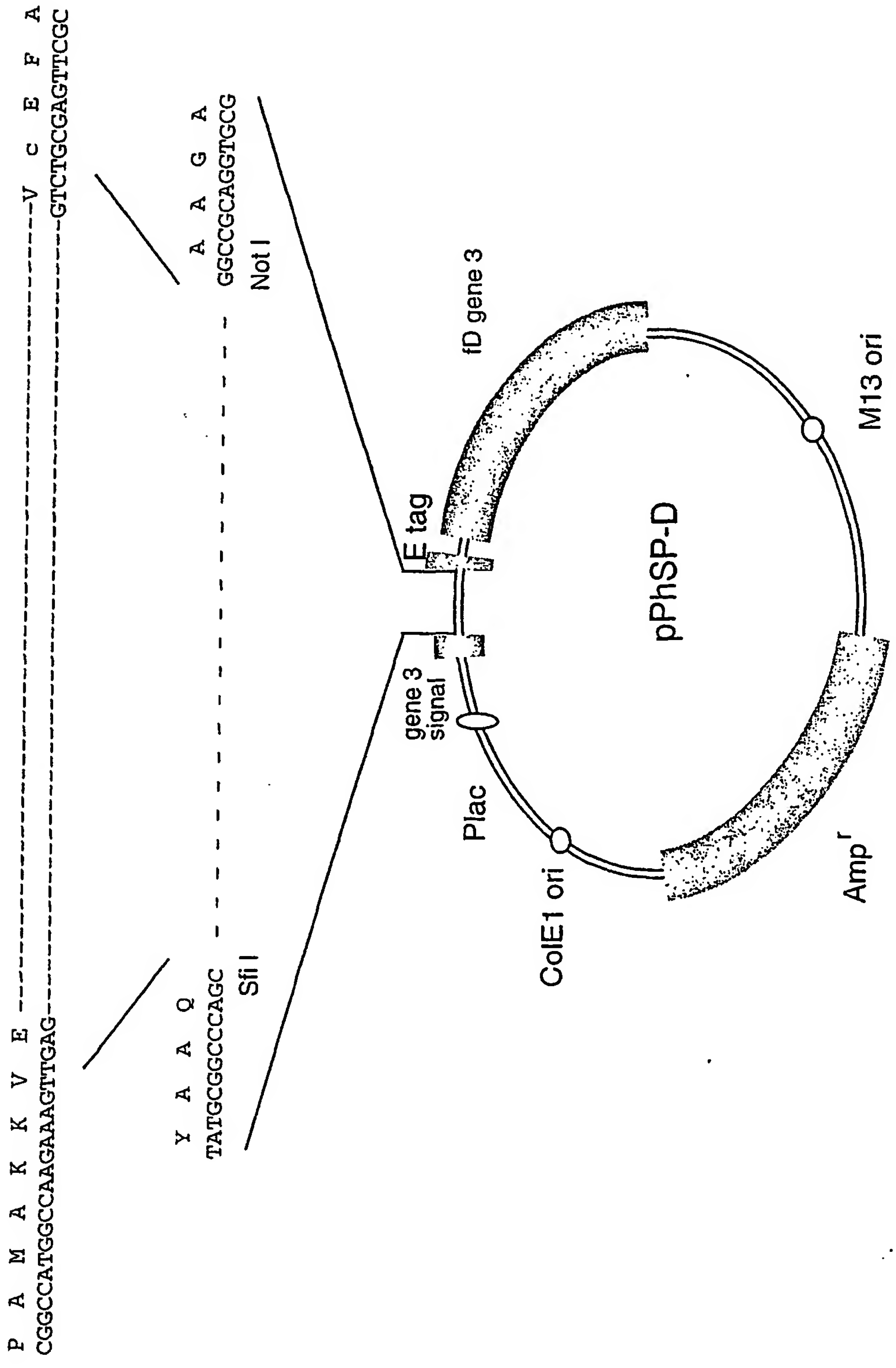
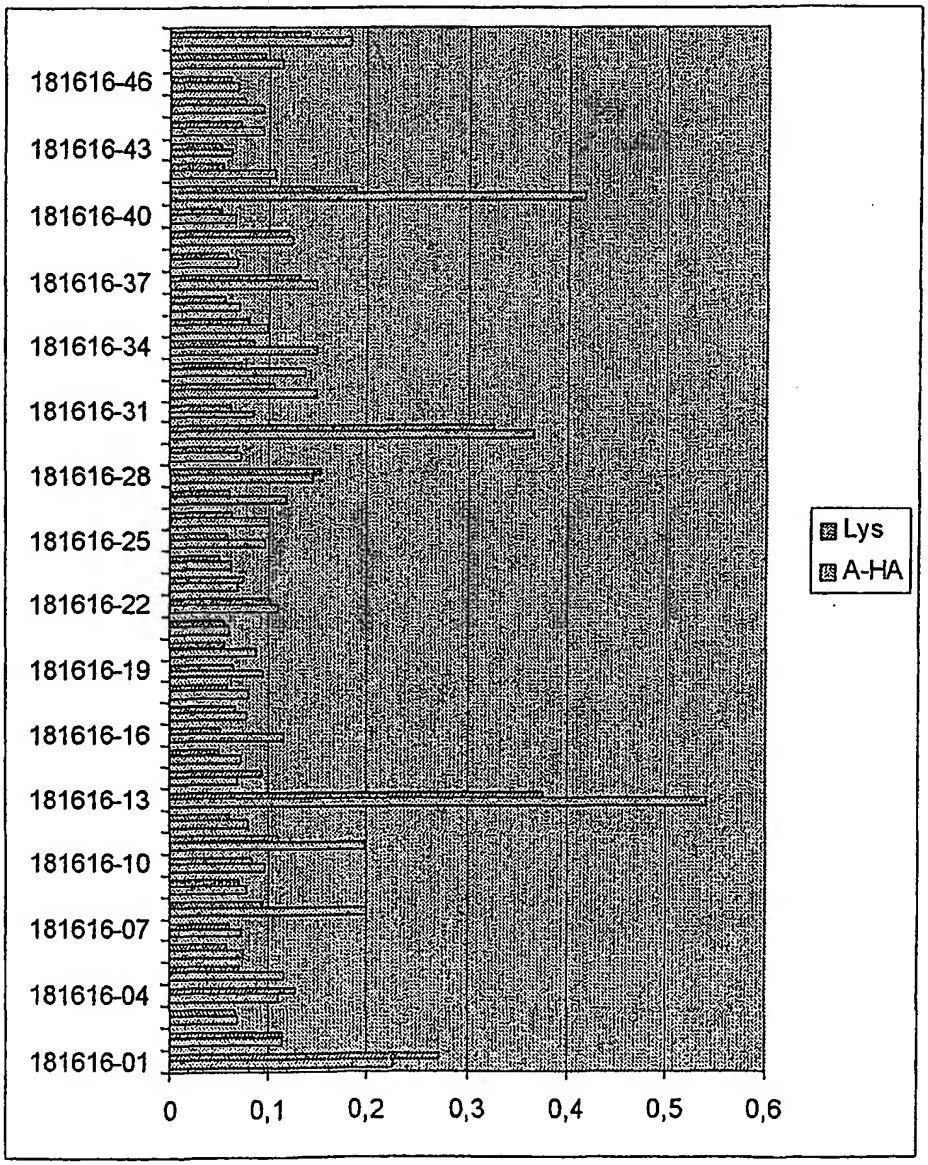


Fig.33

Figure 34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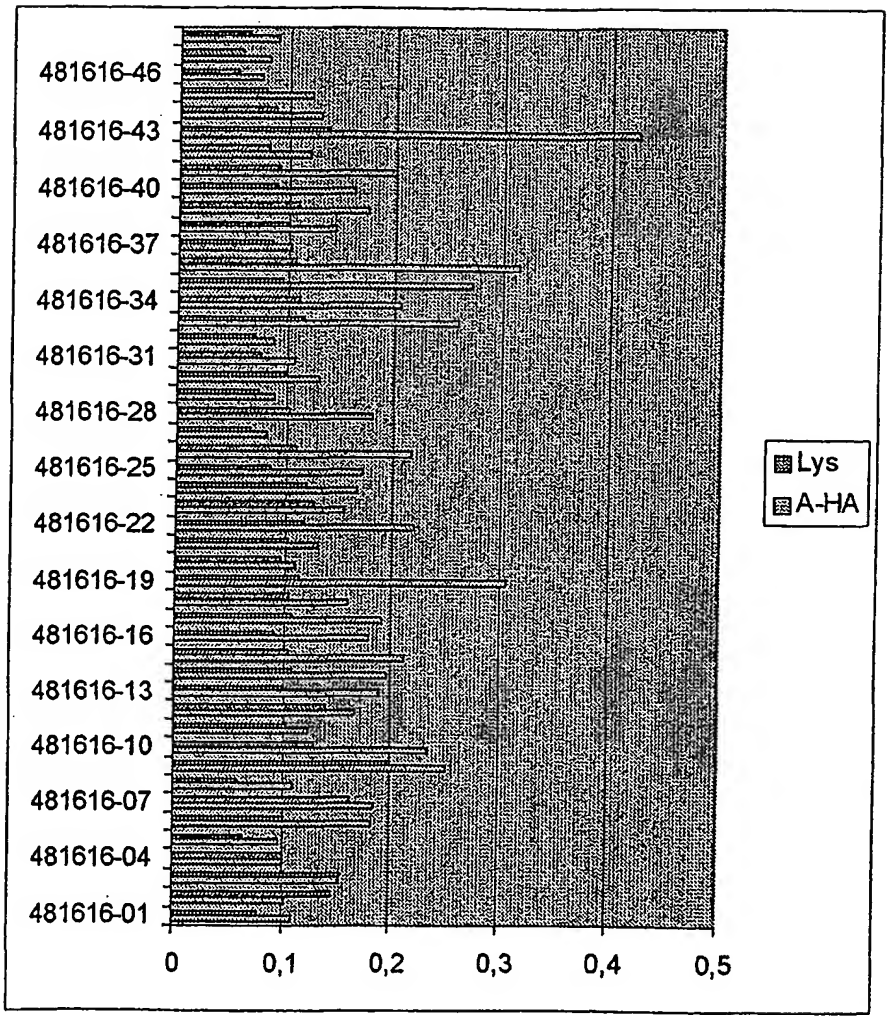
0,5
0,6
Lys
A-HA

Figure 35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Lys
A-HA

SEQUENCE LISTING

<110> Borean Pharma A/S

<120> Combinatorial libraries of proteins having the scaffold structure of C-type lectin-like domains

<130> P200001100 WO JNy

<140>
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<150> DK PA 2000 01872
<151> 2000-12-13

<160> 91

<170> PatentIn Ver. 2.1

<210> 1
<211> 571
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(564)
<223> FX-htlec encoding insert

<400> 1

```
gga tcc atc gag ggt agg ggc gag cca cca acc cag aag ccc aag aag   48
Gly Ser Ile Glu Gly Arg Gly Glu Pro Pro Thr Gln Lys Pro Lys Lys
  1               5                  10                  15

att gta aat gcc aag aaa gat gtt gtg aac aca aag atg ttt gag gag   96
Ile Val Asn Ala Lys Lys Asp Val Val Asn Thr Lys Met Phe Glu Glu
             20                  25                  30

ctc aag agc cgt ctg gac acc ctg gcc cag gag gtg gcc ctg ctg aag  144
Leu Lys Ser Arg Leu Asp Thr Leu Ala Gln Glu Val Ala Leu Leu Lys
         35                  40                  45

gag cag cag gcc ctg cag acg gtc gtc ctg aag ggg acc aag gtg cac  192
Glu Gln Gln Ala Leu Gln Thr Val Val Leu Lys Gly Thr Lys Val His
     50                  55                  60

atg aaa gtc ttt ctg gcc ttc acc cag acg aag acc ttc cac gag gcc  240
Met Lys Val Phe Leu Ala Phe Thr Gln Thr Lys Thr Phe His Glu Ala
 65                  70                  75                  80

agc gag gac tgc atc tcg cgc ggg ggc acc ctg agc acc cct cag act  288
Ser Glu Asp Cys Ile Ser Arg Gly Gly Thr Leu Ser Thr Pro Gln Thr
                 85                  90                  95

ggc tcg gag aac gac gcc ctg tat gag tac ctg cgc cag agc gtg ggc  336
Gly Ser Glu Asn Asp Ala Leu Tyr Glu Tyr Leu Arg Gln Ser Val Gly
            100                 105                 110

aac gag gcc gag atc tgg ctg ggc ctc aac gac atg gcg gcc gag ggc  384
Asn Glu Ala Glu Ile Trp Leu Gly Leu Asn Asp Met Ala Ala Glu Gly
        115                 120                 125
```

```
acc tgg gtg gac atg acc ggt acc cgc atc gcc tac aag aac tgg gag   432
Thr Trp Val Asp Met Thr Gly Thr Arg Ile Ala Tyr Lys Asn Trp Glu
    130                 135                 140

act gag atc acc gcg caa ccc gat ggc ggc aag acc gag aac tgc gcg   480
Thr Glu Ile Thr Ala Gln Pro Asp Gly Gly Lys Thr Glu Asn Cys Ala
145                 150                 155                 160

gtc ctg tca ggc gcg gcc aac ggc aag tgg ttc gac aag cgc tgc cgc   528
Val Leu Ser Gly Ala Ala Asn Gly Lys Trp Phe Asp Lys Arg Cys Arg
                165                 170                 175

gat caa ttg ccc tac atc tgc cag ttc ggg atc gtg taagctt           571
Asp Gln Leu Pro Tyr Ile Cys Gln Phe Gly Ile Val
            180                 185
```

<210> 2
<211> 188
<212> PRT
<213> Homo sapiens

<400> 2

```
Gly Ser Ile Glu Gly Arg Gly Glu Pro Pro Thr Gln Lys Pro Lys Lys
  1               5                  10                  15

Ile Val Asn Ala Lys Lys Asp Val Val Asn Thr Lys Met Phe Glu Glu
            20                  25                  30

Leu Lys Ser Arg Leu Asp Thr Leu Ala Gln Glu Val Ala Leu Leu Lys
        35                  40                  45

Glu Gln Gln Ala Leu Gln Thr Val Val Leu Lys Gly Thr Lys Val His
    50                  55                  60

Met Lys Val Phe Leu Ala Phe Thr Gln Thr Lys Thr Phe His Glu Ala
65                  70                  75                  80

Ser Glu Asp Cys Ile Ser Arg Gly Gly Thr Leu Ser Thr Pro Gln Thr
                85                  90                  95

Gly Ser Glu Asn Asp Ala Leu Tyr Glu Tyr Leu Arg Gln Ser Val Gly
            100                 105                 110

Asn Glu Ala Glu Ile Trp Leu Gly Leu Asn Asp Met Ala Ala Glu Gly
        115                 120                 125

Thr Trp Val Asp Met Thr Gly Thr Arg Ile Ala Tyr Lys Asn Trp Glu
    130                 135                 140

Thr Glu Ile Thr Ala Gln Pro Asp Gly Gly Lys Thr Glu Asn Cys Ala
145                 150                 155                 160

Val Leu Ser Gly Ala Ala Asn Gly Lys Trp Phe Asp Lys Arg Cys Arg
                165                 170                 175

Asp Gln Leu Pro Tyr Ile Cys Gln Phe Gly Ile Val
            180                 185
```

<210> 3
<211> 436
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (1)..(429)
<223> FX-htCTLD encoding insert

<400> 3
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Gly Ser Ile Glu Gly Arg Ala Leu Gln Thr Val Val Leu Lys Gly Thr
1 5 10 15

aag gtg cac atg aaa gtc ttt ctg gcc ttc acc cag acg aag acc ttc 96
Lys Val His Met Lys Val Phe Leu Ala Phe Thr Gln Thr Lys Thr Phe
20 25 30

cac gag gcc agc gag gac tgc atc tcg cgc ggg ggc acc ctg agc acc 144
His Glu Ala Ser Glu Asp Cys Ile Ser Arg Gly Gly Thr Leu Ser Thr
35 40 45

cct cag act ggc tcg gag aac gac gcc ctg tat gag tac ctg cgc cag 192
Pro Gln Thr Gly Ser Glu Asn Asp Ala Leu Tyr Glu Tyr Leu Arg Gln
50 55 60

agc gtg ggc aac gag gcc gag atc tgg ctg ggc ctc aac gac atg gcg 240
Ser Val Gly Asn Glu Ala Glu Ile Trp Leu Gly Leu Asn Asp Met Ala
65 70 75 80

gcc gag ggc acc tgg gtg gac atg acc ggt acc cgc atc gcc tac aag 288
Ala Glu Gly Thr Trp Val Asp Met Thr Gly Thr Arg Ile Ala Tyr Lys
85 90 95

aac tgg gag act gag atc acc gcg caa ccc gat ggc ggc aag acc gag 336
Asn Trp Glu Thr Glu Ile Thr Ala Gln Pro Asp Gly Gly Lys Thr Glu
100 105 110

aac tgc gcg gtc ctg tca ggc gcg gcc aac ggc aag tgg ttc gac aag 384
Asn Cys Ala Val Leu Ser Gly Ala Ala Asn Gly Lys Trp Phe Asp Lys
115 120 125

cgc tgc cgc gat caa ttg ccc tac atc tgc cag ttc ggg atc gtg 429
Arg Cys Arg Asp Gln Leu Pro Tyr Ile Cys Gln Phe Gly Ile Val
130 135 140

taagctt 436

<210> 4
<211> 143
<212> PRT
<213> Homo sapiens

<400> 4
Gly Ser Ile Glu Gly Arg Ala Leu Gln Thr Val Val Leu Lys Gly Thr
1 5 10 15

Lys Val His Met Lys Val Phe Leu Ala Phe Thr Gln Thr Lys Thr Phe
20 25 30

His Glu Ala Ser Glu Asp Cys Ile Ser Arg Gly Gly Thr Leu Ser Thr
35 40 45

Pro Gln Thr Gly Ser Glu Asn Asp Ala Leu Tyr Glu Tyr Leu Arg Gln
50 55 60

Ser Val Gly Asn Glu Ala Glu Ile Trp Leu Gly Leu Asn Asp Met Ala
65 70 75 80

Ala Glu Gly Thr Trp Val Asp Met Thr Gly Thr Arg Ile Ala Tyr Lys
85 90 95

Asn Trp Glu Thr Glu Ile Thr Ala Gln Pro Asp Gly Gly Lys Thr Glu
100 105 110

Asn Cys Ala Val Leu Ser Gly Ala Ala Asn Gly Lys Trp Phe Asp Lys
115 120 125

Arg Cys Arg Asp Gln Leu Pro Tyr Ile Cys Gln Phe Gly Ile Val
130 135 140

<210> 5
<211> 47
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 5
cggctgagcg gcccagccgg ccatggccga gccaccaacc cagaagc 47

<210> 6
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 6
cctgcggccg ccacgatccc gaactgg 27

<210> 7
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 7
cggctgagcg gcccagccgg ccatggccgc cctgcagacg gtc 43

<210> 8
<211> 570
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (8)..(565)
<223> PhTN encoding insert

<400> 8

```
ggcccag ccg gcc atg gcc gag cca cca acc cag aag ccc aag aag att   49
        Pro Ala Met Ala Glu Pro Pro Thr Gln Lys Pro Lys Lys Ile
          1               5                  10

gta aat gcc aag aaa gat gtt gtg aac aca aag atg ttt gag gag ctc   97
Val Asn Ala Lys Lys Asp Val Val Asn Thr Lys Met Phe Glu Glu Leu
 15                  20                  25                  30

aag agc cgt ctg gac acc ctg gcc cag gag gtg gcc ctg ctg aag gag   145
Lys Ser Arg Leu Asp Thr Leu Ala Gln Glu Val Ala Leu Leu Lys Glu
                 35                  40                  45

cag cag gcc ctg cag acg gtc tgc ctg aag ggg acc aag gtg cac atg   193
Gln Gln Ala Leu Gln Thr Val Cys Leu Lys Gly Thr Lys Val His Met
             50                  55                  60

aaa tgc ttt ctg gcc ttc acc cag acg aag acc ttc cac gag gcc agc   241
Lys Cys Phe Leu Ala Phe Thr Gln Thr Lys Thr Phe His Glu Ala Ser
         65                  70                  75

gag gac tgc atc tcg cgc ggg ggc acc ctg agc acc cct cag act ggc   289
Glu Asp Cys Ile Ser Arg Gly Gly Thr Leu Ser Thr Pro Gln Thr Gly
     80                  85                  90

tcg gag aac gac gcc ctg tat gag tac ctg cgc cag agc gtg ggc aac   337
Ser Glu Asn Asp Ala Leu Tyr Glu Tyr Leu Arg Gln Ser Val Gly Asn
 95                  100                 105                 110

gag gcc gag atc tgg ctg ggc ctc aac gac atg gcg gcc gag ggc acc   385
Glu Ala Glu Ile Trp Leu Gly Leu Asn Asp Met Ala Ala Glu Gly Thr
                115                 120                 125

tgg gtg gac atg acc ggc gcc cgc atc gcc tac aag aac tgg gag act   433
Trp Val Asp Met Thr Gly Ala Arg Ile Ala Tyr Lys Asn Trp Glu Thr
            130                 135                 140

gag atc acc gcg caa ccc gat ggc ggc aag acc gag aac tgc gcg gtc   481
Glu Ile Thr Ala Gln Pro Asp Gly Gly Lys Thr Glu Asn Cys Ala Val
        145                 150                 155

ctg tca ggc gcg gcc aac ggc aag tgg ttc gac aag cgc tgc cgc gat   529
Leu Ser Gly Ala Ala Asn Gly Lys Trp Phe Asp Lys Arg Cys Arg Asp
    160                 165                 170

cag ctg ccc tac atc tgc cag ttc ggg atc gtg gcg gccgc             570
Gln Leu Pro Tyr Ile Cys Gln Phe Gly Ile Val Ala
175                 180                 185
```

<210> 9
<211> 186
<212> PRT
<213> Homo sapiens

<400> 9
Pro Ala Met Ala Glu Pro Pro Thr Gln Lys Pro Lys Lys Ile Val Asn
1 5 10 15

Ala Lys Lys Asp Val Val Asn Thr Lys Met Phe Glu Glu Leu Lys Ser
20 25 30

Arg Leu Asp Thr Leu Ala Gln Glu Val Ala Leu Leu Lys Glu Gln Gln
35 40 45

Ala Leu Gln Thr Val Cys Leu Lys Gly Thr Lys Val His Met Lys Cys
50 55 60

Phe Leu Ala Phe Thr Gln Thr Lys Thr Phe His Glu Ala Ser Glu Asp
65 70 75 80

Cys Ile Ser Arg Gly Gly Thr Leu Ser Thr Pro Gln Thr Gly Ser Glu
85 90 95

Asn Asp Ala Leu Tyr Glu Tyr Leu Arg Gln Ser Val Gly Asn Glu Ala
100 105 110

Glu Ile Trp Leu Gly Leu Asn Asp Met Ala Ala Glu Gly Thr Trp Val
115 120 125

Asp Met Thr Gly Ala Arg Ile Ala Tyr Lys Asn Trp Glu Thr Glu Ile
130 135 140

Thr Ala Gln Pro Asp Gly Gly Lys Thr Glu Asn Cys Ala Val Leu Ser
145 150 155 160

Gly Ala Ala Asn Gly Lys Trp Phe Asp Lys Arg Cys Arg Asp Gln Leu
165 170 175

Pro Tyr Ile Cys Gln Phe Gly Ile Val Ala
180 185

<210> 10
<211> 438
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (8)..(433)
<223> PhTN3 encoding insert

<400> 10
ggcccag ccg gcc atg gcc gcc ctg cag acg gtc tgc ctg aag ggg acc 49
Pro Ala Met Ala Ala Leu Gln Thr Val Cys Leu Lys Gly Thr
1 5 10

aag gtg cac atg aaa tgc ttt ctg gcc ttc acc cag acg aag acc ttc 97
Lys Val His Met Lys Cys Phe Leu Ala Phe Thr Gln Thr Lys Thr Phe
15 20 25 30

```
cac gag gcc agc gag gac tgc atc tcg cgc ggg ggc acc ctg agc acc   145
His Glu Ala Ser Glu Asp Cys Ile Ser Arg Gly Gly Thr Leu Ser Thr
                35                  40                  45

cct cag act ggc tcg gag aac gac gcc ctg tat gag tac ctg cgc cag   193
Pro Gln Thr Gly Ser Glu Asn Asp Ala Leu Tyr Glu Tyr Leu Arg Gln
            50                  55                  60

agc gtg ggc aac gag gcc gag atc tgg ctg ggc ctc aac gac atg gcg   241
Ser Val Gly Asn Glu Ala Glu Ile Trp Leu Gly Leu Asn Asp Met Ala
        65                  70                  75

gcc gag ggc acc tgg gtg gac atg acc ggc gcc cgc atc gcc tac aag   289
Ala Glu Gly Thr Trp Val Asp Met Thr Gly Ala Arg Ile Ala Tyr Lys
    80                  85                  90

aac tgg gag act gag atc acc gcg caa ccc gat ggc ggc aag acc gag   337
Asn Trp Glu Thr Glu Ile Thr Ala Gln Pro Asp Gly Gly Lys Thr Glu
95                  100                 105                 110

aac tgc gcg gtc ctg tca ggc gcg gcc aac ggc aag tgg ttc gac aag   385
Asn Cys Ala Val Leu Ser Gly Ala Ala Asn Gly Lys Trp Phe Asp Lys
                115                 120                 125

cgc tgc cgc gat cag ctg ccc tac atc tgc cag ttc ggg atc gtg gcg   433
Arg Cys Arg Asp Gln Leu Pro Tyr Ile Cys Gln Phe Gly Ile Val Ala
            130                 135                 140

gccgc                                                             438
```

<210> 11
<211> 142
<212> PRT
<213> Homo sapiens

<400> 11

```
Pro Ala Met Ala Ala Leu Gln Thr Val Cys Leu Lys Gly Thr Lys Val
  1               5                  10                  15

His Met Lys Cys Phe Leu Ala Phe Thr Gln Thr Lys Thr Phe His Glu
            20                  25                  30

Ala Ser Glu Asp Cys Ile Ser Arg Gly Gly Thr Leu Ser Thr Pro Gln
        35                  40                  45

Thr Gly Ser Glu Asn Asp Ala Leu Tyr Glu Tyr Leu Arg Gln Ser Val
    50                  55                  60

Gly Asn Glu Ala Glu Ile Trp Leu Gly Leu Asn Asp Met Ala Ala Glu
65                  70                  75                  80

Gly Thr Trp Val Asp Met Thr Gly Ala Arg Ile Ala Tyr Lys Asn Trp
                85                  90                  95

Glu Thr Glu Ile Thr Ala Gln Pro Asp Gly Gly Lys Thr Glu Asn Cys
            100                 105                 110

Ala Val Leu Ser Gly Ala Ala Asn Gly Lys Trp Phe Asp Lys Arg Cys
        115                 120                 125
```

```
Arg Asp Gln Leu Pro Tyr Ile Cys Gln Phe Gly Ile Val Ala
    130                 135                 140
```

<210> 12
<211> 570
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (8)..(565)
<223> Phtlec encoding insert

<400> 12

```
ggcccag ccg gcc atg gcc gag cca cca acc cag aag ccc aag aag att   49
        Pro Ala Met Ala Glu Pro Pro Thr Gln Lys Pro Lys Lys Ile
          1               5                  10

gta aat gcc aag aaa gat gtt gtg aac aca aag atg ttt gag gag ctc   97
Val Asn Ala Lys Lys Asp Val Val Asn Thr Lys Met Phe Glu Glu Leu
 15                  20                  25                  30

aag agc cgt ctg gac acc ctg gcc cag gag gtg gcc ctg ctg aag gag   145
Lys Ser Arg Leu Asp Thr Leu Ala Gln Glu Val Ala Leu Leu Lys Glu
                35                  40                  45

cag cag gcc ctg cag acg gtc gtc ctg aag ggg acc aag gtg cac atg   193
Gln Gln Ala Leu Gln Thr Val Val Leu Lys Gly Thr Lys Val His Met
            50                  55                  60

aaa gtc ttt ctg gcc ttc acc cag acg aag acc ttc cac gag gcc agc   241
Lys Val Phe Leu Ala Phe Thr Gln Thr Lys Thr Phe His Glu Ala Ser
        65                  70                  75

gag gac tgc atc tcg cgc ggg ggc acc ctg agc acc cct cag act ggc   289
Glu Asp Cys Ile Ser Arg Gly Gly Thr Leu Ser Thr Pro Gln Thr Gly
    80                  85                  90

tcg gag aac gac gcc ctg tat gag tac ctg cgc cag agc gtg ggc aac   337
Ser Glu Asn Asp Ala Leu Tyr Glu Tyr Leu Arg Gln Ser Val Gly Asn
 95                  100                 105                 110

gag gcc gag atc tgg ctg ggc ctc aac gac atg gcg gcc gag ggc acc   385
Glu Ala Glu Ile Trp Leu Gly Leu Asn Asp Met Ala Ala Glu Gly Thr
                115                 120                 125

tgg gtg gac atg acc ggt acc cgc atc gcc tac aag aac tgg gag act   433
Trp Val Asp Met Thr Gly Thr Arg Ile Ala Tyr Lys Asn Trp Glu Thr
            130                 135                 140

gag atc acc gcg caa ccc gat ggc ggc aag acc gag aac tgc gcg gtc   481
Glu Ile Thr Ala Gln Pro Asp Gly Gly Lys Thr Glu Asn Cys Ala Val
        145                 150                 155

ctg tca ggc gcg gcc aac ggc aag tgg ttc gac aag cgc tgc cgc gat   529
Leu Ser Gly Ala Ala Asn Gly Lys Trp Phe Asp Lys Arg Cys Arg Asp
    160                 165                 170
```

caa ttg ccc tac atc tgc cag ttc ggg atc gtg gcg gccgc 570
Gln Leu Pro Tyr Ile Cys Gln Phe Gly Ile Val Ala
175 180 185

<210> 13
<211> 186
<212> PRT
<213> Homo sapiens

<400> 13
Pro Ala Met Ala Glu Pro Pro Thr Gln Lys Pro Lys Lys Ile Val Asn
1 5 10 15

Ala Lys Lys Asp Val Val Asn Thr Lys Met Phe Glu Glu Leu Lys Ser
20 25 30

Arg Leu Asp Thr Leu Ala Gln Glu Val Ala Leu Leu Lys Glu Gln Gln
35 40 45

Ala Leu Gln Thr Val Val Leu Lys Gly Thr Lys Val His Met Lys Val
50 55 60

Phe Leu Ala Phe Thr Gln Thr Lys Thr Phe His Glu Ala Ser Glu Asp
65 70 75 80

Cys Ile Ser Arg Gly Gly Thr Leu Ser Thr Pro Gln Thr Gly Ser Glu
85 90 95

Asn Asp Ala Leu Tyr Glu Tyr Leu Arg Gln Ser Val Gly Asn Glu Ala
100 105 110

Glu Ile Trp Leu Gly Leu Asn Asp Met Ala Ala Glu Gly Thr Trp Val
115 120 125

Asp Met Thr Gly Thr Arg Ile Ala Tyr Lys Asn Trp Glu Thr Glu Ile
130 135 140

Thr Ala Gln Pro Asp Gly Gly Lys Thr Glu Asn Cys Ala Val Leu Ser
145 150 155 160

Gly Ala Ala Asn Gly Lys Trp Phe Asp Lys Arg Cys Arg Asp Gln Leu
165 170 175

Pro Tyr Ile Cys Gln Phe Gly Ile Val Ala
180 185

<210> 14
<211> 438
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (8)..(433)
<223> PhtCTLD encoding insert

<400> 14

```
ggcccag ccg gcc atg gcc gcc ctg cag acg gtc gtc ctg aag ggg acc   49
        Pro Ala Met Ala Ala Leu Gln Thr Val Val Leu Lys Gly Thr
          1               5                  10

aag gtg cac atg aaa gtc ttt ctg gcc ttc acc cag acg aag acc ttc   97
Lys Val His Met Lys Val Phe Leu Ala Phe Thr Gln Thr Lys Thr Phe
 15                  20                  25                  30

cac gag gcc agc gag gac tgc atc tcg cgc ggg ggc acc ctg agc acc   145
His Glu Ala Ser Glu Asp Cys Ile Ser Arg Gly Gly Thr Leu Ser Thr
                 35                  40                  45

cct cag act ggc tcg gag aac gac gcc ctg tat gag tac ctg cgc cag   193
Pro Gln Thr Gly Ser Glu Asn Asp Ala Leu Tyr Glu Tyr Leu Arg Gln
             50                  55                  60

agc gtg ggc aac gag gcc gag atc tgg ctg ggc ctc aac gac atg gcg   241
Ser Val Gly Asn Glu Ala Glu Ile Trp Leu Gly Leu Asn Asp Met Ala
         65                  70                  75

gcc gag ggc acc tgg gtg gac atg acc ggt acc cgc atc gcc tac aag   289
Ala Glu Gly Thr Trp Val Asp Met Thr Gly Thr Arg Ile Ala Tyr Lys
     80                  85                  90

aac tgg gag act gag atc acc gcg caa ccc gat ggc ggc aag acc gag   337
Asn Trp Glu Thr Glu Ile Thr Ala Gln Pro Asp Gly Gly Lys Thr Glu
 95                  100                 105                 110

aac tgc gcg gtc ctg tca ggc gcg gcc aac ggc aag tgg ttc gac aag   385
Asn Cys Ala Val Leu Ser Gly Ala Ala Asn Gly Lys Trp Phe Asp Lys
                115                 120                 125

cgc tgc cgc gat caa ttg ccc tac atc tgc cag ttc ggg atc gtg gcg   433
Arg Cys Arg Asp Gln Leu Pro Tyr Ile Cys Gln Phe Gly Ile Val Ala
            130                 135                 140

gccgc                                                             438
```

<210> 15
<211> 142
<212> PRT
<213> Homo sapiens

<400> 15

```
Pro Ala Met Ala Ala Leu Gln Thr Val Val Leu Lys Gly Thr Lys Val
  1               5                  10                  15

His Met Lys Val Phe Leu Ala Phe Thr Gln Thr Lys Thr Phe His Glu
             20                  25                  30

Ala Ser Glu Asp Cys Ile Ser Arg Gly Gly Thr Leu Ser Thr Pro Gln
         35                  40                  45

Thr Gly Ser Glu Asn Asp Ala Leu Tyr Glu Tyr Leu Arg Gln Ser Val
     50                  55                  60

Gly Asn Glu Ala Glu Ile Trp Leu Gly Leu Asn Asp Met Ala Ala Glu
 65                  70                  75                  80
```

Gly Thr Trp Val Asp Met Thr Gly Thr Arg Ile Ala Tyr Lys Asn Trp
85 90 95

Glu Thr Glu Ile Thr Ala Gln Pro Asp Gly Gly Lys Thr Glu Asn Cys
100 105 110

Ala Val Leu Ser Gly Ala Ala Asn Gly Lys Trp Phe Asp Lys Arg Cys
115 120 125

Arg Asp Gln Leu Pro Tyr Ile Cys Gln Phe Gly Ile Val Ala
130 135 140

<210> 16
<211> 555
<212> DNA
<213> Mus musculus

<220>
<223> EcoRI to HindIII insert containing mtlec encoding part

<400> 16
ggaattcgag tcacccactc ccaaggccaa gaaggctgca aatgccaaga aagatttggt 60
gagctcaaag atgtcgagga gctcaagaac aggatggatg tcctggccca ggaggtggcc 120
ctgctgaagg agaagcaggc cttacagact gtggtcctga agggcaccaa ggtgaacttg 180
aaggtcctcc tggccttcac ccaaccgaag accttccatg aggcgagcga ggactgcatc 240
tcgcaagggg gcacgctggg caccccgcag tcagagctag agaacgaggc gctgttcgag 300
tacgcgcgcc acagcgtggg caacgatgcg gagatctggc tgggcctcaa cgacatggcc 360
gcggaaggcg cctgggtgga catgaccggt accctcctgg cctacaagaa ctgggagacg 420
gagatcacga cgcaacccga cggcggcaaa gccgagaact gcgccgccct gtctggcgca 480
gccaacggca agtggttcga caagcgatgc cgcgatcaat tgccctacat ctgccagttt 540
gccattgtga agctt 555

<210> 17
<211> 77
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: oligonucleotide

<400> 17
cggaattcga gtcacccact cccaaggcca agaaggctgc aaatgccaag aaagatttgg 60
tgagctcaaa gatgttc 77

<210> 18
<211> 94
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: oligonucleotide

<400> 18
gcggatccag gcctgcttct ccttcagcag ggccacctcc tgggccagga catccatcct 60
gttcttgagc tcctcgaaca tctttgagct cacc 94

<210> 19
<211> 97
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 19
gcaggcctta cagactgtgt gcctgaaggg caccaaggtg aacttgaagt gcctcctggc 60
cttcacccaa ccgaagacct tccatgaggc gagcgag 97

<210> 20
<211> 93
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 20
ccgcatgctt cgaacagcgc ctcgttctct agctctgact gcggggtgcc cagcgtgccc 60
ccttgcgaga tgcagtcctc gctcgcctca tgg 93

<210> 21
<211> 61
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 21
ggttcgaata cgcgcgccac agcgtgggca acgatgcgga gatctaaatg ctcccaattg 60
c 61

<210> 22
<211> 55
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 22
ccaagcttca caatggcaaa ctggcagatg tagggcaatt gggagcattt agatc 55

<210> 23
<211> 86
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 23
cggagatctg gctgggcctc aacgacatgg ccgcggaagg cgcctgggtg gacatgaccg 60
gtaccctcct ggcctacaag aactgg 86

<210> 24
<211> 130
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 24
gggcaattga tcgcggcatc gcttgtcgaa cctcttgccg ttggctgcgc cagacagggc 60
ggcgcagttc tcggctttgc cgccgtcggg ttgcgtcgtg atctccgtct cccagttctt 120
gtaggccagg 130

<210> 25
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 25
ctgggatcca tccagggtcg cgagtcaccc actcccaagg 40

<210> 26
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 26
ccgaagctta cacaatggca aactggc 27

<210> 27
<211> 39
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 27
ctgggatcca tccagggtcg cgccttacag actgtggtc 39

<210> 28
<211> 568
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(561)
<223> FX-mtlec encoding insert

<400> 28

```
gga tcc atc cag ggt cgc gag tca ccc act ccc aag gcc aag aag gct   48
Gly Ser Ile Gln Gly Arg Glu Ser Pro Thr Pro Lys Ala Lys Lys Ala
  1               5                  10                  15

gca aat gcc aag aaa gat ttg gtg agc tca aag atg ttc gag gag ctc   96
Ala Asn Ala Lys Lys Asp Leu Val Ser Ser Lys Met Phe Glu Glu Leu
             20                  25                  30

aag aac agg atg gat gtc ctg gcc cag gag gtg gcc ctg ctg aag gag   144
Lys Asn Arg Met Asp Val Leu Ala Gln Glu Val Ala Leu Leu Lys Glu
         35                  40                  45

aag cag gcc tta cag act gtg gtc ctg aag ggc acc aag gtg aac ttg   192
Lys Gln Ala Leu Gln Thr Val Val Leu Lys Gly Thr Lys Val Asn Leu
     50                  55                  60

aag gtc ctc ctg gcc ttc acc caa ccg aag acc ttc cat gag gcg agc   240
Lys Val Leu Leu Ala Phe Thr Gln Pro Lys Thr Phe His Glu Ala Ser
 65                  70                  75                  80

gag gac tgc atc tcg caa ggg ggc acg ctg ggc acc ccg cag tca gag   288
Glu Asp Cys Ile Ser Gln Gly Gly Thr Leu Gly Thr Pro Gln Ser Glu
                 85                  90                  95

cta gag aac gag gcg ctg ttc gag tac gcg cgc cac agc gtg ggc aac   336
Leu Glu Asn Glu Ala Leu Phe Glu Tyr Ala Arg His Ser Val Gly Asn
            100                 105                 110

gat gcg gag atc tgg ctg ggc ctc aac gac atg gcc gcg gaa ggc gcc   384
Asp Ala Glu Ile Trp Leu Gly Leu Asn Asp Met Ala Ala Glu Gly Ala
        115                 120                 125

tgg gtg gac atg acc ggt acc ctc ctg gcc tac aag aac tgg gag acg   432
Trp Val Asp Met Thr Gly Thr Leu Leu Ala Tyr Lys Asn Trp Glu Thr
    130                 135                 140

gag atc acg acg caa ccc gac ggc ggc aaa gcc gag aac tgc gcc gcc   480
Glu Ile Thr Thr Gln Pro Asp Gly Gly Lys Ala Glu Asn Cys Ala Ala
145                 150                 155                 160

ctg tct ggc gca gcc aac ggc aag tgg ttc gac aag cga tgc cgc gat   528
Leu Ser Gly Ala Ala Asn Gly Lys Trp Phe Asp Lys Arg Cys Arg Asp
                165                 170                 175

caa ttg ccc tac atc tgc cag ttt gcc att gtg taagctt               568
Gln Leu Pro Tyr Ile Cys Gln Phe Ala Ile Val
            180                 185
```

<210> 29
<211> 187
<212> PRT
<213> Mus musculus

<400> 29
Gly Ser Ile Gln Gly Arg Glu Ser Pro Thr Pro Lys Ala Lys Lys Ala
1 5 10 15

Ala Asn Ala Lys Lys Asp Leu Val Ser Ser Lys Met Phe Glu Glu Leu
20 25 30

Lys Asn Arg Met Asp Val Leu Ala Gln Glu Val Ala Leu Leu Lys Glu
35 40 45

Lys Gln Ala Leu Gln Thr Val Val Leu Lys Gly Thr Lys Val Asn Leu
50 55 60

Lys Val Leu Leu Ala Phe Thr Gln Pro Lys Thr Phe His Glu Ala Ser
65 70 75 80

Glu Asp Cys Ile Ser Gln Gly Gly Thr Leu Gly Thr Pro Gln Ser Glu
85 90 95

Leu Glu Asn Glu Ala Leu Phe Glu Tyr Ala Arg His Ser Val Gly Asn
100 105 110

Asp Ala Glu Ile Trp Leu Gly Leu Asn Asp Met Ala Ala Glu Gly Ala
115 120 125

Trp Val Asp Met Thr Gly Thr Leu Leu Ala Tyr Lys Asn Trp Glu Thr
130 135 140

Glu Ile Thr Thr Gln Pro Asp Gly Gly Lys Ala Glu Asn Cys Ala Ala
145 150 155 160

Leu Ser Gly Ala Ala Asn Gly Lys Trp Phe Asp Lys Arg Cys Arg Asp
165 170 175

Gln Leu Pro Tyr Ile Cys Gln Phe Ala Ile Val
180 185

<210> 30
<211> 436
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)..(429)
<223> FX-mtCTLD encoding insert

<400> 30
gga tcc atc cag ggt cgc gcc tta cag act gtg gtc ctg aag ggc acc 48
Gly Ser Ile Gln Gly Arg Ala Leu Gln Thr Val Val Leu Lys Gly Thr
1 5 10 15

aag gtg aac ttg aag gtc ctc ctg gcc ttc acc caa ccg aag acc ttc 96
Lys Val Asn Leu Lys Val Leu Leu Ala Phe Thr Gln Pro Lys Thr Phe
20 25 30

```
cat gag gcg agc gag gac tgc atc tcg caa ggg ggc acg ctg ggc acc   144
His Glu Ala Ser Glu Asp Cys Ile Ser Gln Gly Gly Thr Leu Gly Thr
         35                  40                  45

ccg cag tca gag cta gag aac gag gcg ctg ttc gag tac gcg cgc cac   192
Pro Gln Ser Glu Leu Glu Asn Glu Ala Leu Phe Glu Tyr Ala Arg His
     50                  55                  60

agc gtg ggc aac gat gcg gag atc tgg ctg ggc ctc aac gac atg gcc   240
Ser Val Gly Asn Asp Ala Glu Ile Trp Leu Gly Leu Asn Asp Met Ala
 65                  70                  75                  80

gcg gaa ggc gcc tgg gtg gac atg acc ggt acc ctc ctg gcc tac aag   288
Ala Glu Gly Ala Trp Val Asp Met Thr Gly Thr Leu Leu Ala Tyr Lys
                 85                  90                  95

aac tgg gag acg gag atc acg acg caa ccc gac ggc ggc aaa gcc gag   336
Asn Trp Glu Thr Glu Ile Thr Thr Gln Pro Asp Gly Gly Lys Ala Glu
            100                 105                 110

aac tgc gcc gcc ctg tct ggc gca gcc aac ggc aag tgg ttc gac aag   384
Asn Cys Ala Ala Leu Ser Gly Ala Ala Asn Gly Lys Trp Phe Asp Lys
        115                 120                 125

cga tgc cgc gat caa ttg ccc tac atc tgc cag ttt gcc att gtg       429
Arg Cys Arg Asp Gln Leu Pro Tyr Ile Cys Gln Phe Ala Ile Val
    130                 135                 140

taagctt                                                          436
```

<210> 31
<211> 143
<212> PRT
<213> Mus musculus

<400> 31

```
Gly Ser Ile Gln Gly Arg Ala Leu Gln Thr Val Val Leu Lys Gly Thr
  1               5                  10                  15

Lys Val Asn Leu Lys Val Leu Leu Ala Phe Thr Gln Pro Lys Thr Phe
             20                  25                  30

His Glu Ala Ser Glu Asp Cys Ile Ser Gln Gly Gly Thr Leu Gly Thr
         35                  40                  45

Pro Gln Ser Glu Leu Glu Asn Glu Ala Leu Phe Glu Tyr Ala Arg His
     50                  55                  60

Ser Val Gly Asn Asp Ala Glu Ile Trp Leu Gly Leu Asn Asp Met Ala
 65                  70                  75                  80

Ala Glu Gly Ala Trp Val Asp Met Thr Gly Thr Leu Leu Ala Tyr Lys
                 85                  90                  95

Asn Trp Glu Thr Glu Ile Thr Thr Gln Pro Asp Gly Gly Lys Ala Glu
            100                 105                 110

Asn Cys Ala Ala Leu Ser Gly Ala Ala Asn Gly Lys Trp Phe Asp Lys
        115                 120                 125
```

Arg Cys Arg Asp Gln Leu Pro Tyr Ile Cys Gln Phe Ala Ile Val
130 135 140

<210> 32
<211> 47
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 32
cggctgagcg gcccagccgg ccatggccga gtcacccact cccaagg 47

<210> 33
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 33
cctgcggccg ccacgatccc gaactgg 27

<210> 34
<211> 46
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 34
cggctgagcg gcccagccgg ccatggccgc ttacagactg gtggtc 46

<210> 35
<211> 570
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (8)..(565)
<223> Pmtlec encoding insert

<400> 35
ggcccag ccg gcc atg gcc gag tca ccc act ccc aag gcc aag aag gct 49
Pro Ala Met Ala Glu Ser Pro Thr Pro Lys Ala Lys Lys Ala
1 5 10

gca aat gcc aag aaa gat ttg gtg agc tca aag atg ttc gag gag ctc 97
Ala Asn Ala Lys Lys Asp Leu Val Ser Ser Lys Met Phe Glu Glu Leu
15 20 25 30

```
aag aac agg atg gat gtc ctg gcc cag gag gtg gcc ctg ctg aag gag   145
Lys Asn Arg Met Asp Val Leu Ala Gln Glu Val Ala Leu Leu Lys Glu
                35                  40                  45

aag cag gcc tta cag act gtg gtc ctg aag ggc acc aag gtg aac ttg   193
Lys Gln Ala Leu Gln Thr Val Val Leu Lys Gly Thr Lys Val Asn Leu
            50                  55                  60

aag gtc ctc ctg gcc ttc acc caa ccg aag acc ttc cat gag gcg agc   241
Lys Val Leu Leu Ala Phe Thr Gln Pro Lys Thr Phe His Glu Ala Ser
        65                  70                  75

gag gac tgc atc tcg caa ggg ggc acg ctg ggc acc ccg cag tca gag   289
Glu Asp Cys Ile Ser Gln Gly Gly Thr Leu Gly Thr Pro Gln Ser Glu
    80                  85                  90

cta gag aac gag gcg ctg ttc gag tac gcg cgc cac agc gtg ggc aac   337
Leu Glu Asn Glu Ala Leu Phe Glu Tyr Ala Arg His Ser Val Gly Asn
95                  100                 105                 110

gat gcg gag atc tgg ctg ggc ctc aac gac atg gcc gcg gaa ggc gcc   385
Asp Ala Glu Ile Trp Leu Gly Leu Asn Asp Met Ala Ala Glu Gly Ala
                115                 120                 125

tgg gtg gac atg acc ggt acc ctc ctg gcc tac aag aac tgg gag acg   433
Trp Val Asp Met Thr Gly Thr Leu Leu Ala Tyr Lys Asn Trp Glu Thr
            130                 135                 140

gag atc acg acg caa ccc gac ggc ggc aaa gcc gag aac tgc gcc gcc   481
Glu Ile Thr Thr Gln Pro Asp Gly Gly Lys Ala Glu Asn Cys Ala Ala
        145                 150                 155

ctg tct ggc gca gcc aac ggc aag tgg ttc gac aag cga tgc cgc gat   529
Leu Ser Gly Ala Ala Asn Gly Lys Trp Phe Asp Lys Arg Cys Arg Asp
    160                 165                 170

caa ttg ccc tac atc tgc cag ttt gcc att gtg gcg gccgc             570
Gln Leu Pro Tyr Ile Cys Gln Phe Ala Ile Val Ala
175                 180                 185
```

<210> 36
<211> 186
<212> PRT
<213> Mus musculus

<400> 36

```
Pro Ala Met Ala Glu Ser Pro Thr Pro Lys Ala Lys Lys Ala Ala Asn
  1               5                  10                  15

Ala Lys Lys Asp Leu Val Ser Ser Lys Met Phe Glu Glu Leu Lys Asn
            20                  25                  30

Arg Met Asp Val Leu Ala Gln Glu Val Ala Leu Leu Lys Glu Lys Gln
        35                  40                  45

Ala Leu Gln Thr Val Val Leu Lys Gly Thr Lys Val Asn Leu Lys Val
    50                  55                  60

Leu Leu Ala Phe Thr Gln Pro Lys Thr Phe His Glu Ala Ser Glu Asp
65                  70                  75                  80
```

```
Cys Ile Ser Gln Gly Gly Thr Leu Gly Thr Pro Gln Ser Glu Leu Glu
        85                  90                  95

Asn Glu Ala Leu Phe Glu Tyr Ala Arg His Ser Val Gly Asn Asp Ala
           100                 105                 110

Glu Ile Trp Leu Gly Leu Asn Asp Met Ala Ala Glu Gly Ala Trp Val
       115                 120                 125

Asp Met Thr Gly Thr Leu Leu Ala Tyr Lys Asn Trp Glu Thr Glu Ile
    130                 135                 140

Thr Thr Gln Pro Asp Gly Gly Lys Ala Glu Asn Cys Ala Ala Leu Ser
145                 150                 155                 160

Gly Ala Ala Asn Gly Lys Trp Phe Asp Lys Arg Cys Arg Asp Gln Leu
                165                 170                 175

Pro Tyr Ile Cys Gln Phe Ala Ile Val Ala
           180                 185
```

<210> 37
<211> 438
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (8)..(433)
<223> PmtCTLD encoding insert

<400> 37

```
ggcccag ccg gcc atg gcc gcc tta cag act gtg gtc ctg aag ggc acc   49
        Pro Ala Met Ala Ala Leu Gln Thr Val Val Leu Lys Gly Thr
          1               5                  10

aag gtg aac ttg aag gtc ctc ctg gcc ttc acc caa ccg aag acc ttc   97
Lys Val Asn Leu Lys Val Leu Leu Ala Phe Thr Gln Pro Lys Thr Phe
 15                  20                  25                  30

cat gag gcg agc gag gac tgc atc tcg caa ggg ggc acg ctg ggc acc  145
His Glu Ala Ser Glu Asp Cys Ile Ser Gln Gly Gly Thr Leu Gly Thr
                35                  40                  45

ccg cag tca gag cta gag aac gag gcg ctg ttc gag tac gcg cgc cac  193
Pro Gln Ser Glu Leu Glu Asn Glu Ala Leu Phe Glu Tyr Ala Arg His
            50                  55                  60

agc gtg ggc aac gat gcg gag atc tgg ctg ggc ctc aac gac atg gcc  241
Ser Val Gly Asn Asp Ala Glu Ile Trp Leu Gly Leu Asn Asp Met Ala
         65                  70                  75

gcg gaa ggc gcc tgg gtg gac atg acc ggt acc ctc ctg gcc tac aag  289
Ala Glu Gly Ala Trp Val Asp Met Thr Gly Thr Leu Leu Ala Tyr Lys
     80                  85                  90

aac tgg gag acg gag atc acg acg caa ccc gac ggc ggc aaa gcc gag  337
Asn Trp Glu Thr Glu Ile Thr Thr Gln Pro Asp Gly Gly Lys Ala Glu
 95                  100                 105                 110
```

```
aac tgc gcc gcc ctg tct ggc gca gcc aac ggc aag tgg ttc gac aag   385
Asn Cys Ala Ala Leu Ser Gly Ala Ala Asn Gly Lys Trp Phe Asp Lys
                115                 120                 125

cga tgc cgc gat caa ttg ccc tac atc tgc cag ttt gcc att gtg gcg   433
Arg Cys Arg Asp Gln Leu Pro Tyr Ile Cys Gln Phe Ala Ile Val Ala
            130                 135                 140

gccgc                                                             438
```

<210> 38
<211> 142
<212> PRT
<213> Mus musculus

<400> 38

```
Pro Ala Met Ala Ala Leu Gln Thr Val Val Leu Lys Gly Thr Lys Val
  1               5                  10                  15

Asn Leu Lys Val Leu Leu Ala Phe Thr Gln Pro Lys Thr Phe His Glu
             20                  25                  30

Ala Ser Glu Asp Cys Ile Ser Gln Gly Gly Thr Leu Gly Thr Pro Gln
         35                  40                  45

Ser Glu Leu Glu Asn Glu Ala Leu Phe Glu Tyr Ala Arg His Ser Val
     50                  55                  60

Gly Asn Asp Ala Glu Ile Trp Leu Gly Leu Asn Asp Met Ala Ala Glu
 65                  70                  75                  80

Gly Ala Trp Val Asp Met Thr Gly Thr Leu Leu Ala Tyr Lys Asn Trp
                 85                  90                  95

Glu Thr Glu Ile Thr Thr Gln Pro Asp Gly Gly Lys Ala Glu Asn Cys
            100                 105                 110

Ala Ala Leu Ser Gly Ala Ala Asn Gly Lys Trp Phe Asp Lys Arg Cys
        115                 120                 125

Arg Asp Gln Leu Pro Tyr Ile Cys Gln Phe Ala Ile Val Ala
    130                 135                 140
```

<210> 39
<211> 116
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 39

```
cgcctacaag aactggnnsn nsnnsnnsnn snnscaaccc gatnnsnnsn nsnnsgagaa 60
ctgcgcggtc ctgtcaggcg cggccaacgg caagtggnns gacaagcgct gccgcg     116
```

<210> 40
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 40
gaccggtacc cgcatcgcct acaagaactg g 31

<210> 41
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 41
gtagggcaat tgatcgcggc agcgcttgtc 30

<210> 42
<211> 94
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 42
gctgggcctc aacgacnnsn nsnnsgagnn snnstgggtg gacatgaccg gtacccgcat 60
cgcctacaag aactgggaga ctgagatcac cgcg 94

<210> 43
<211> 102
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 43
cgcggcagcg cttgtcgaac cacttgccgt tggccgcgcc tgacaggacc gcgcagttct 60
csnnsnnsnn snnatcgggt tgcgcggtga tctcagtctc cc 102

<210> 44
<211> 31
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
oligonucleotide

<400> 44
cgaggccgag atctggctgg gcctcaacga c 31

<210> 45
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 45
gggcaacgag gccgagatct ggctgggcct c 31

<210> 46
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 46
cctgaccctg cagcgcttg 19

<210> 47
<211> 81
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 47
cgagatctgg ctgggcctca acgacnnsnn snnsnnsnns nnsgagggca cctgggtgga 60
catgaccggt acccgcatcg c 81

<210> 48
<211> 78
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 48
cgagatctgg ctgggcctca acgacnnsnn snnsnnsnns gagggcacct gggtggacat 60
gaccggtacc cgcatcgc 78

<210> 49
<211> 94
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 49
gctgggcctc aacgacnnsn nsnnsgagnn snnstgggtg gacatgaccg gtacccgcat 60
cgcctacaag aactgggaga ctgagatcac cgcg 94

<210> 50
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 50
gcgatgcggg taccggtc 18

<210> 51
<211> 89
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 51
gcatcgccta caagaactgg gagactgaga tcaccgcgca acccgatggc ggcnnsnnsn 60
nsnnsnnsnn sgagaactgc gcggtcctg 89

<210> 52
<211> 86
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 52
gcatcgccta caagaactgg gagactgaga tcaccgcgca acccgatggc ggcnnsnnsn 60
nsnnsnnsga gaactgcgcg gtcctg 86

<210> 53
<211> 34
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:
oligonucleotide

<400> 53
catgaccggt acccgcatcg cctacaagaa ctgg 34

<210> 54
<211> 66
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 54
cctgaccctg cagcgcttgt cgaaccactt gccgttggcc gcgcctgaca ggaccgcgca 60
gttctc 66

<210> 55
<211> 45
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:
oligonucleotide

<400> 55
ggtacctaag tgacgatatc ctgacctaac tgcagggatc aattg 45

<210> 56
<211> 343
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (8)..(274)
<223> Human PhtCPB insert

<400> 56
ggcccag ccg gcc atg gcc gcc ctc cag acg gtc tgc ctg aag ggg acc 49
Pro Ala Met Ala Ala Leu Gln Thr Val Cys Leu Lys Gly Thr
1 5 10

aag gtg cac atg aaa tgc ttt ctg gcc ttc acc cag acg aag acc ttc 97
Lys Val His Met Lys Cys Phe Leu Ala Phe Thr Gln Thr Lys Thr Phe
15 20 25 30

cac gag gcc agc gag gac tgc atc tcg cgc ggg ggc acc ctg agc acc 145
His Glu Ala Ser Glu Asp Cys Ile Ser Arg Gly Gly Thr Leu Ser Thr
35 40 45

cct cag act ggc tcg gag aac gac gcc ctg tat gag tac ctg cgc cag 193
Pro Gln Thr Gly Ser Glu Asn Asp Ala Leu Tyr Glu Tyr Leu Arg Gln
50 55 60

```
agc gtg ggc aac gag gcc gag atc tgg ctg ggc ctc aac gac atg gcg   241
Ser Val Gly Asn Glu Ala Glu Ile Trp Leu Gly Leu Asn Asp Met Ala
         65                  70                  75

gcc gag ggc acc tgg gtg gac atg acc ggt acc taagtgacga tatcctgacc 294
Ala Glu Gly Thr Trp Val Asp Met Thr Gly Thr
     80                  85

taactgcagg gatcaattgc cctacatctg ccagttcggg atcgtgtag             343
```

<210> 57
<211> 89
<212> PRT
<213> Homo sapiens

<400> 57

```
Pro Ala Met Ala Ala Leu Gln Thr Val Cys Leu Lys Gly Thr Lys Val
  1               5                  10                  15

His Met Lys Cys Phe Leu Ala Phe Thr Gln Thr Lys Thr Phe His Glu
             20                  25                  30

Ala Ser Glu Asp Cys Ile Ser Arg Gly Gly Thr Leu Ser Thr Pro Gln
         35                  40                  45

Thr Gly Ser Glu Asn Asp Ala Leu Tyr Glu Tyr Leu Arg Gln Ser Val
     50                  55                  60

Gly Asn Glu Ala Glu Ile Trp Leu Gly Leu Asn Asp Met Ala Ala Glu
 65                  70                  75                  80

Gly Thr Trp Val Asp Met Thr Gly Thr
                 85
```

<210> 58
<211> 405
<212> DNA
<213> Rattus rattus

<220>
<221> CDS
<222> (8)..(400)
<223> Rat PrMBP insert

<400> 58

```
ggcccag ccg gcc atg gcc aac aag ttg cat gcc ttc tcc atg ggt aaa   49
        Pro Ala Met Ala Asn Lys Leu His Ala Phe Ser Met Gly Lys
          1               5                  10

aag tct ggg aag aag ttc ttt gtg acc aac cat gaa agg atg ccc ttt   97
Lys Ser Gly Lys Lys Phe Phe Val Thr Asn His Glu Arg Met Pro Phe
 15                  20                  25                  30

tcc aaa gtc aag gcc ctg tgc tca gag ctc cga ggc act gtg gct atc   145
Ser Lys Val Lys Ala Leu Cys Ser Glu Leu Arg Gly Thr Val Ala Ile
                 35                  40                  45
```

```
ccc aag aat gct gag gag aac aag gcc atc caa gaa gtg gct aaa acc   193
Pro Lys Asn Ala Glu Glu Asn Lys Ala Ile Gln Glu Val Ala Lys Thr
            50                  55                  60

tct gcc ttc cta ggc atc acg gac gag gtg act gaa ggc caa ttc atg   241
Ser Ala Phe Leu Gly Ile Thr Asp Glu Val Thr Glu Gly Gln Phe Met
        65                  70                  75

tat gtg aca ggg ggg agg ctc acc tac agc aac tgg aaa aag gat gag   289
Tyr Val Thr Gly Gly Arg Leu Thr Tyr Ser Asn Trp Lys Lys Asp Glu
    80                  85                  90

ccc aat gac cat ggc tct ggg gaa gac tgt gtc act ata gta gac aac   337
Pro Asn Asp His Gly Ser Gly Glu Asp Cys Val Thr Ile Val Asp Asn
 95                 100                 105                 110

ggt ctg tgg aat gac atc tcc tgc caa gct tcc cac acg gct gtc tgc   385
Gly Leu Trp Asn Asp Ile Ser Cys Gln Ala Ser His Thr Ala Val Cys
                115                 120                 125

gag ttc cca gcc gcg gccgc                                        405
Glu Phe Pro Ala Ala
            130
```

<210> 59
<211> 131
<212> PRT
<213> Rattus rattus

<400> 59

```
Pro Ala Met Ala Asn Lys Leu His Ala Phe Ser Met Gly Lys Lys Ser
  1               5                  10                  15

Gly Lys Lys Phe Phe Val Thr Asn His Glu Arg Met Pro Phe Ser Lys
            20                  25                  30

Val Lys Ala Leu Cys Ser Glu Leu Arg Gly Thr Val Ala Ile Pro Lys
        35                  40                  45

Asn Ala Glu Glu Asn Lys Ala Ile Gln Glu Val Ala Lys Thr Ser Ala
    50                  55                  60

Phe Leu Gly Ile Thr Asp Glu Val Thr Glu Gly Gln Phe Met Tyr Val
 65                  70                  75                  80

Thr Gly Gly Arg Leu Thr Tyr Ser Asn Trp Lys Lys Asp Glu Pro Asn
                85                  90                  95

Asp His Gly Ser Gly Glu Asp Cys Val Thr Ile Val Asp Asn Gly Leu
            100                 105                 110

Trp Asn Asp Ile Ser Cys Gln Ala Ser His Thr Ala Val Cys Glu Phe
        115                 120                 125

Pro Ala Ala
    130
```

<210> 60
<211> 408
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (8)..(403)
<223> Human PhSP-D insert

<400> 60
ggcccag ccg gcc atg gcc aag aaa gtt gag ctc ttc cca aat ggc caa 49
Pro Ala Met Ala Lys Lys Val Glu Leu Phe Pro Asn Gly Gln
1 5 10

agt gtg ggg gag aag att ttc aag acą gca ggc ttt gta aaa cca ttt 97
Ser Val Gly Glu Lys Ile Phe Lys Thr Ala Gly Phe Val Lys Pro Phe
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acg gag gca cag ctg ctg tgc aca cag gct ggt gga cag ttg gcc tct 145
Thr Glu Ala Gln Leu Leu Cys Thr Gln Ala Gly Gly Gln Leu Ala Ser
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cca cgc tct gcc gct gag aat gcc gcc ttg caa cag ctg gtc gta gct 193
Pro Arg Ser Ala Ala Glu Asn Ala Ala Leu Gln Gln Leu Val Val Ala
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aag aac gag gct gct ttc ctg agc atg act gat tcc aag aca gag ggc 241
Lys Asn Glu Ala Ala Phe Leu Ser Met Thr Asp Ser Lys Thr Glu Gly
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aag ttc acc tac ccc aca gga gag tcc ctg gtc tat tcc aac tgg gcc 289
Lys Phe Thr Tyr Pro Thr Gly Glu Ser Leu Val Tyr Ser Asn Trp Ala
80 85 90

cca ggg gag ccc aac gat gat ggc ggg tca gag gac tgt gtg gag atc 337
Pro Gly Glu Pro Asn Asp Asp Gly Gly Ser Glu Asp Cys Val Glu Ile
95 100 105 110

ttc acc aat ggc aag tgg aat gac agg gct tgt gga gaa aag cgt ctt 385
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115 120 125

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<213> Homo sapiens

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35 40 45

Ser Ala Ala Glu Asn Ala Ala Leu Gln Gln Leu Val Val Ala Lys Asn
50 55 60

Glu Ala Ala Phe Leu Ser Met Thr Asp Ser Lys Thr Glu Gly Lys Phe
65 70 75 80

Thr Tyr Pro Thr Gly Glu Ser Leu Val Tyr Ser Asn Trp Ala Pro Gly
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Glu Pro Asn Asp Asp Gly Gly Ser Glu Asp Cys Val Glu Ile Phe Thr
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Cys Glu Phe Ala
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<400> 63
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<210> 67
<211> 68
<212> DNA
<213> Artificial Sequence

<220>
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<210> 68
<211> 62
<212> DNA
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<220>
<223> Description of Artificial Sequence:
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<210> 69
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<210> 72
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<210> 73
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<223> Description of Artificial Sequence:
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<400> 73
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<210> 74
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<400> 74
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<210> 75
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<212> DNA
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<220>
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<210> 76
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<210> 80
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<210> 81
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<213> Artificial Sequence

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<210> 82
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<213> Artificial Sequence

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<400> 82
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<210> 83
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<400> 83
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<210> 84
<211> 77
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<210> 85
<211> 83
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<210> 86
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<400> 90
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60/272,098 28 February 2001 (28.02.2001) US

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(74) Agent: **HOFMAN-BANG ZACCO A/S**; Hans Bekkevolds Allé 7, DK-2900 Hellerup (DK).

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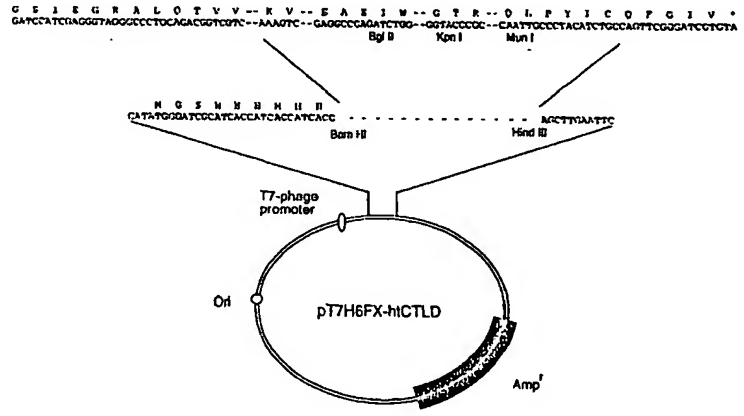
[Continued on next page]

(54) Title: COMBINATORIAL LIBRARIES OF PROTEINS HAVING THE SCAFFOLD STRUCTURE OF C-TYPE LECTIN-LIKE DOMAINS



(57) Abstract: A novel family of protein libraries comprising CTLDS (C-type Lectin-Like Domains) in which internal polypeptide loop-regions lining the ligand binding sites in CTLDs have been replaced with ensembles of completely or partially randomised polypeptide segments. Tetranectin CTLDs were chosen as framework for the preferred embodiment of the invention; and versatile phagemid vectors useful in the generation and manipulation of human and murine tetranectin CTLD libraries are disclosed as part of this invention. Tetranectin CTLDs in monomeric as well as in trimeric form are efficiently displayed as gene III fusions in fully functional form by the recombinant fd phage display vector. CTLD derivatives with affinity for new ligands may readily be isolated from libraries of vectors displaying CTLDs, in which loop-regions have been randomised, using one or more rounds of enrichment by screening or selection followed by amplification of the enriched subpopulation in each round. The efficiency with which protein products containing CTLDs with new binding properties can be produced, e.g. by bacterial expression in *in vitro* refolding, in mono-, tri-, or multimeric formats provides important advantages in terms of simplicity, cost and efficiency of generation, production and diagnostic or therapeutic applications in comparison to recombinant antibody derivatives.

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Published:
— *with international search report*

(88) Date of publication of the international search report:
9 January 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

national Application No
PCT/DK 01/00825

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C12N15/10 C12N15/62 C07K14/47

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
WPI Data, PAJ, CAB Data, SEQUENCE SEARCH, BIOSIS, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GRAVERSEN JONAS HEILSKOV ET AL: "Mutational analysis of affinity and selectivity of kringle-tetranectin interaction. Grafting novel kringle affinity onto the tetranectin lectin scaffold." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 275, no. 48, 1 December 2000 (2000-12-01), pages 37390-37396, XP002175478 ISSN: 0021-9258 cited in the application the whole document --- -/--	1-4,6,7, 12,13, 39,58

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 August 2002	27/08/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hornig, H

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	GRAVERSEN JONAS HEILSKOV ET AL: "The plasminogen binding site of the C-type lectin tetranectin is located in the carbohydrate recognition domain, and binding is sensitive to both calcium and lysine." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 44, 30 October 1998 (1998-10-30), pages 29241-29246, XP002175479 ISSN: 0021-9258 cited in the application the whole document	1-4,6,7, 12,13, 39,58
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INTERNATIONAL SEARCH REPORT

National Application No
PCT/DK 01/00825

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	DOI N ET AL: "SCREENING OF CONFORMATIONALLY CONSTRAINED RANDOM POLYPEPTIDE LIBRARIES DISPLAYED ON A PROTEIN SCAFFOLD" CMLS CELLULAR AND MOLECULAR LIFE SCIENCES, BIRKHAUSER VERLAG, BASEL, CH, vol. 54, no. 5, May 1998 (1998-05), pages 394-404, XP000891835 ISSN: 1420-682X the whole document	
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A	WO 00 20574 A (RIGEL PHARMACEUTICALS INC) 13 April 2000 (2000-04-13) the whole document	
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Information on patent family members

national Application No
PCT/DK 01/00825

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